

THE ULTRASTRUCTURAL ORGANIZATION
OF THE COLUMNAR ABSORBING CELLS
OF THE MOUSE JEJUNUM

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*An electron microscopic study
including some experiments regarding the
problem of fixation and an investigation
of Vitamin A deficiency*

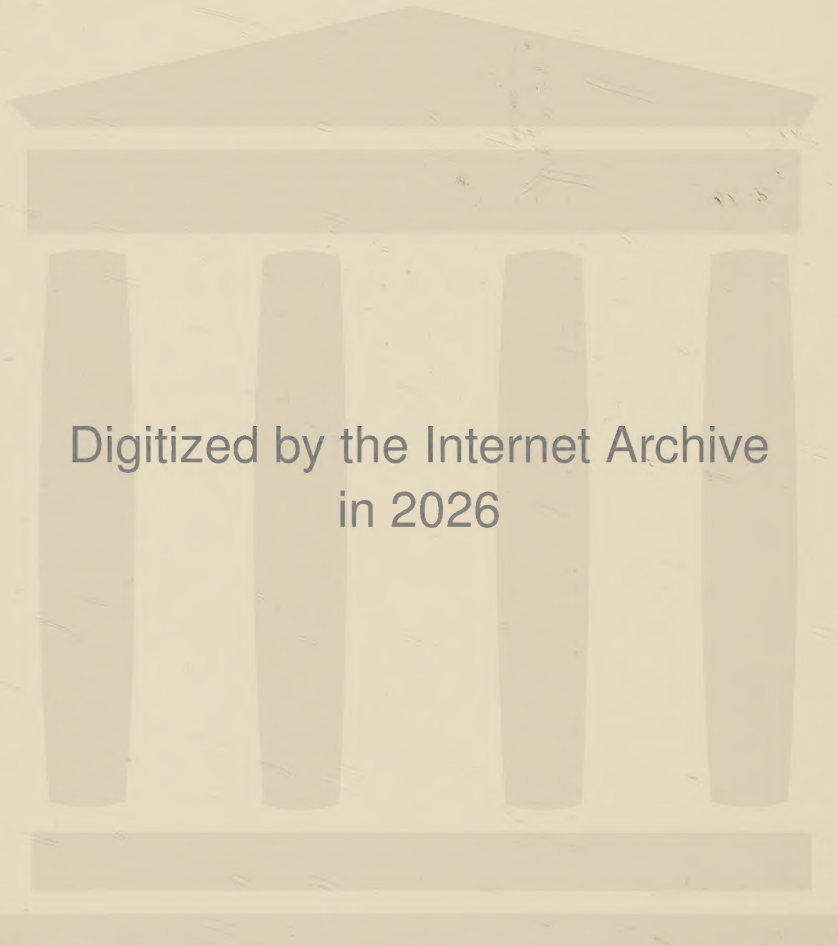
BY

HANS ZETTERQVIST

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FROM THE DEPARTMENT OF ANATOMY, KAROLINSKA INSTITUTET,
STOCKHOLM, SWEDEN.

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To my wife

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PREFACE

This study has been carried out in the Laboratory for Biological Ultrastructure Research in the Department of Anatomy at Karolinska Institutet, Stockholm, under the direction of the head of this laboratory, Doctor FRITIOF SJÖSTRAND, Associate Professor of Anatomy. Throughout the investigation he has given me invaluable encouragement and has spent much time with me in very stimulating discussions regarding the work. He has also given many useful suggestions with regard to the presentation of the material.

The head of the Department of Anatomy, Professor TURE PETRÉN, has kindly placed at my disposal the resources of the department. He has also followed my investigations with great interest.

The head of the Vitamin Department at the National Institute of Public Health, Stockholm, Professor E. BRUNIUS, has given valuable advice regarding the vitamin experiments and has also furnished me with some necessary substances for the diet.

Mr. STAFFAN EKBLOM of the Statistical Research Group at Stockholm's Högskola has aided me with the statistical evaluation of the material.

Miss ULLA FOGELBERG has shown an unfailing interest and through her skillful technical assistance has been of the greatest help to me.

Through her drawings and help in photographic enlargements, Miss MAJ BERGHMAN has also contributed to this study.

Mrs. GRETA SARGEANT has done the translation and Dr. and Mrs. R. BERGMAN and Dr. H. SHELDON have given help with the technical terms.

To all these and all others who in different ways have kindly assisted me in producing this work I wish to express my deep gratitude.

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Stockholm, January 1956.

HANS ZETTERQVIST

GENERAL INTRODUCTION

The main part of the absorption of food-stuff takes place through the single cell layer which forms the epithelium of the intestine. Consequently, the cells which form this epithelium have been given much attention in morphological as well as physiological studies. This is evident from the numerous investigations which have been published in the last one hundred years.

The resolution of the light microscope is too limited to make possible an analysis of the fine structure of various cell components, including those of primary importance for a detailed understanding of the pathways for molecular transportation through the epithelium from the lumen of the intestine to the lymph and blood vessels of the subepithelial connective tissue.

With the development of methods for ultrathin sectioning of tissues the morphologic study of cell ultrastructure may now be performed by means of electron microscopy. Even when the technique was rather imperfect, according to the standards of today, it was possible to show that the cross striation which had been observed in the free border of the intestinal epithelial cells was caused by closely packed cytoplasmic projections of fine dimensions.

As a result of the research in the last few years technical advancements for ultrathin sectioning has made it possible to study the structure of cells on a supramolecular level. It is now possible, for example, to show the relationship of the plasma membrane to the brush border projections and also to elucidate the fine structure of this membrane.

The purpose of the present work has been to study the ultrastructural organization of the absorbing cells of the intestinal epithelium of mice in order to map the absorption pathways through the epithelium. The various cell organelles that are more or less directly involved in the mechanism of molecular transportation through the cells have been analyzed.

Since it is known that fixation procedures often result in structural artefacts, an analysis has been performed of specimens fixed in solutions of different osmolar concentrations, of different pH and at different times after death.

A study of intestinal epithelium from vitamin A deficient mice has been carried out in order to determine if any structural changes occur that would correspond to the assumed general effect of vitamin A deficiency on epithelial tissue.

MATERIAL AND METHODS

The animals used in the experiments were adult white mice weighing 15 to 25 grams, which had been starved for 12 hours and had been without water for 4 hours. To prevent coprophagy they were kept on wire netting in a glass jar. The animals were killed by decapitation at a temperature of $+1^{\circ}$ to $+2^{\circ}\text{C}$, after which the abdomen was quickly opened and the proximal jejunum was found, tracing from the stomach. A piece of intestine a few cm. in length was removed and cut opened lengthwise. Strips about 1 mm. in width were then cut with a razor blade knife and placed directly in 1 % osmium tetroxide solution buffered to pH 7.2—7.4 (PALADE 1952 a) with veronal-acetate buffer (MICHAELIS 1931) and rendered isotonic by the addition of sodium chloride, potassium chloride and calcium chloride (SJÖSTRAND 1953 a, 1954). (See below.)

The entire procedure from the decapitation to the immersion of the specimen in the osmium solution took, as a rule, less than 2 minutes. The specimens were then treated according to the following scheme:

Osmium tetroxide 1 %, pH 7.2—7.4, isotonic 2—4 hrs			
Tyrodé's solution (balanced)		$\frac{1}{2}$ —1 hr	3—4 changes
Ethyl alcohol 70 %	over-night or	$\frac{1}{2}$ —1 hr	3—4 changes
„ „ 95 %		$\frac{1}{2}$ —1 hr	3—4 changes
„ „ absolute		1—2 hrs	3—4 changes

The specimens were kept in a cold room during the whole of this procedure, with the exception of the last change of absolute alcohol which was done at room temperature. From absolute alcohol the specimens were transferred to a 10:1 mixture of n-butyl-methyl methacrylate (NEWMAN, BORYSKO and SWERDLOW 1949) containing 0.1—0.5 % benzoyl peroxide as a catalyst. After 3 to 4 changes in this solution the specimens were placed in gelatin capsules containing a partially polymerized methacrylate mixture and were polymerized at 45°C . After 24 hours at this temperature the desired hardness of the plastic was usually obtained.

Under the dissecting microscope the surrounding plastic was removed so that the surface of the villus was exposed in the apex of a four-sided pyramid. The angle at the top varied between 70° and 90° .

Sectioning was done with the microtome designed by SJÖSTRAND¹ and

¹ Manufactured by LKB-Produkter Fabr. AB, Box 12085, Stockholm, Sweden.

with specially polished razor blades mounted in a curved position (SjöSTRAND 1951). The sections were collected from a 20 % ethyl alcohol solution on formvar coated copper grids.

The sections were examined without removing the plastic in a RCA EMU 2 c electron microscope with compensated objective pole piece, an objective aperture of 50 μ , with three apertures in the projector lens and reduced condensor lens aperture.

Magnification was calibrated by means of diffraction grating and Dow latex spheres.

The isotonic buffer solution for fixation was prepared in accordance with the directions given by MICHAELIS (1931) and modified by SJÖSTRAND (1954):

Stock Buffer Solution A:	Sodium acetate	9.714 gm.
	Veronal-sodium	14.714 gm.
	Distilled water to make	500 ml.

Stock Buffer Solution B:	Sodium chloride	40.25 gm.
	Potassium chloride	2.1 gm.
	Calcium chloride	0.9 gm.
	Distilled water to make	500 ml.

The isotonic fixative solution buffered to pH 7.2—7.4 will then be of the following composition:

Solution A	10 ml.
Solution B	3.4 ml.
HCl 0.1 n	11 ml.
Distilled water to make	50 ml.
Osmium tetroxide	0.5 gm.

The tonicity of this solution was estimated to be 0.34 M. The freezing-point depression of this solution is 0.59°C; this slightly exceeds that for blood containing 5 % CO₂ at 0.55°C (EVANS 1945).

In analyzing the effect of fixing solutions of varying osmolar concentration, the following compositions were used:

Hypotonic solution.

Solution A	10 ml.
Solution B	—
HCl 0.1 n	11 ml.
Distilled water to make	50 ml.
Osmium tetroxide	0.5 gm.

This solution has an estimated osmolar concentration of 0.14 M. The pH is 7.2—7.4.

Hypertonic solution.

Solution A	10 ml.
Solution B	7.25 ml.
HCl 0.1 n	10 ml.
Distilled water to make	50 ml.
Osmium tetroxide	0.5 gm.

The osmolar concentration of this solution is estimated to be 0.56 M. The pH is 7.2—7.4.

In studying the effect of varying pH the following solutions were used:

pH 5.1.

Solution A	10 ml.
Solution B	3 ml.
HCl 0.1 n	17.5 ml.
Distilled water to make	50 ml.
Osmium tetroxide	0.5 gm.

The estimated osmolar concentration of the solution is 0.34 M.

pH 9.5.

Solution A	10 ml.
Solution B	4.15 ml.
HCl 0.1 n	—
Distilled water to make	50 ml.
Osmium tetroxide	0.5 gm.

The estimated osmolar concentration of the solution is 0.34 M.

Electron micrographs were made at original magnifications varying between 1,600 and 26,000 times and enlarged photographically to the desired size.

For determination of finer dimensions, e.g., the plasma membrane and the mitochondrial membranes, prints have been chosen which were taken with an initial magnification greater than 8,500 times and were enlarged photographically up to 5 times. Greater photographic enlargements, as a rule, have not been used in order to avoid the granulation caused by the grain factor of the film, which results in enlargements with less sharp contours.

Measurements were made on the prints with an 8 power magnifying lens equipped with a graduated rule (0.1 mm. graduation).

The resolution achieved in this material is better than 30 Å.

The analysis of variance carried out on the obtained values for the dimensions of the plasma membrane on the free cell surface and for the mitochondrial outer and inner membranes has shown that the most obvious

variance is found between values obtained from different cells of the same animal. The variance between the values obtained from different animals as well as those obtained by measurements of different membranes in the same cell is insignificant (Tab. 1, 2). Therefore in determining the means, standard errors and standard deviations (SD), the cell means have been made the basis for the determinations.

The light micrographs have been made from 0.6 to 3.0 μ thick sections cut with a Spencer microtome from the same osmium-fixed specimens which have been used for electron microscopy. The methacrylate was removed and microscopy performed with phase contrast technique.

Specimens have been taken from 200 animals, of which good fixation has been obtained in 75. From sections of these 75 animals, 1,100 electron-micrographs have been made which form the basis for the present analysis.

II

THE NORMAL ULTRASTRUCTURE OF THE COLUMNAR ABSORBING CELL

THE BRUSH BORDER.

Earlier observations.

In 1837, in *Symbolae ad anatomiam villorum intestinalium*, HENLE described a thin structureless layer forming a border between the cell and the lumen of the small intestine of the rabbit. This was the first recorded observation of what is now known as the "brush border" of the epithelial cells of the small intestine.

"Vibratile bodies" on the intestinal epithelial cell surface of dogs were described by GRUBY and DELAFOND (1843). J. R. BAKER (1942) in interpreting this structure believes that in all probability these authors observed a striated structure.

BRÜCKE (1854) denied the occurrence of a border limiting the free surface of the cell. He considered that the cell, being an open canal, would serve as an intermediate for the transport of substances to the villus parenchyma. Earlier observations suggested that the border could be explained by mucus, an opinion that has gained many followers, among them MOLESCHOTT and MARFELS (1854), WIEGANDT (1860), SCHULTZE (1867), and VON THANHOFFER (1874).

These views advanced by BRÜCKE were opposed by FUNKE (1856) and KÖLLIKER (1856), who independently confirmed HENLE's observations concerning the occurrence of a membrane, and further stated that they had observed a striated structure in this membrane. FUNKE emphasized the resemblance to ciliated epithelium, but he also pointed out the possibility that the structure could be a system of small pores. The latter theory was supported by KÖLLIKER. This view was subsequently shared by WELKER (1857), DONDERS (1857), LEYDIG (1857), FRIEDREICH (1858), BALOGH (1860), SCHNEIDER (1902), and most recently by J. R. BAKER (1942).

The presumption that the striations could be due to post-mortem changes was presented by VON WITTICH (1857), and later by FORTUNATOW (1877).

A division of the border into layers parallel to the cell surface was described by ERDMANN (1867) and EIMER (1868).

In 1857 BRETTAUER and STEINACH published the results of an investigation of the intestinal epithelium in rabbits, guinea-pigs, dogs and man. In this study they advanced, for the first time, the opinion that the striated

structure represented a system of small rod-shaped extensions. In a drawing accompanying their text, 10 to 20 extensions per cell are depicted.

Later, many authors supported this theory of a border of rod-shaped extensions in the intestinal epithelium, although the interpretation of the structure varied. R. HEIDENHAIN (1858, 1888) and KYRKLUND (1886) considered that the rods were extendible protoplasmatic projections, and VON THANHOFFER (1874) observed movements of the rods. ZIPKIN (1903), after investigating conditions in the monkey (*Macacus rhesus*), was able to divide the rod into an "Aussen-" and an "Innenglied", the two parts being separated by a granule. Between the rods, at the same level as the granules, there was a line. The basal part of the "Innenglied" widened, forming the so-called basal ellipsoid. CLARA (1926) confirmed ZIPKIN's observations after studies of birds intestine and also described the occurrence of a ground substance between the rods. BARGMANN and SCHEFFLER (1943) studied the brush border in the epithelium of the small intestine in man and found analogous conditions.

The results of the first electron microscopic investigation of the structure of the brush border were presented by GRANGER and R. F. BAKER (1949, 1950), after studying the epithelium of the small intestine in rats. They demonstrated that the brush border is composed of cylindrical perpendicular projections with rounded apices, which are on the average $0.62\ \mu$ in length and $0.08\ \mu$ in width. The number of projections per cell was estimated at 3,000, and per square millimetre of intestinal surface at 200,000,000. With respect to the cell border and its relation to the extensions, the authors considered that no definite conclusions could be drawn. They observed the lines parallel to the cell surface, which had been described by ZIPKIN, and assumed that the outer line consisted of the cell border, while the inner line was a differentiated cytoplasmic component. The so-called border granule at the transition between the "Aussen-" and the "Innenglied" could not be identified in the EMG, nor could any structural differences between the outer and the inner part of the separate extensions be seen. The extensions were merely said to become somewhat narrower towards the base. As for the conditions in the basal parts of the rod, the authors could not decide whether there was a granule in the basal part of the rod which corresponded to the "basal ellipsoid".

With respect to the functional significance of the extensions, GRANGER and BAKER suggested that they serve to increase the absorbing surface. According to the authors, this increase would be 30 times that of a plane surface.

GRANGER and BAKER were able further to observe a reversed correlation between the length and the width of the extensions and suggested that the

changes in the shape of the brush border units may depend on varying functional states.

Subsequent electron microscopic studies by DALTON, et al., (1950) and DALTON (1951 a, b) confirm the general observations.

After these earliest electron microscopic analyses, J. R. BAKER (1951) published a light microscopic study on the absorption of fat in the intestinal epithelium of mice, in which he reported the occurrence of canals or pores between the brush border extensions through which absorption in the form of particles might take place. The average dimensions of these canals were said to be 0.42μ .

Results.

General description. These investigations on the brush border zone in the intestinal epithelium of the mouse confirm, on the whole, previous observations concerning its gross structure. Unlike earlier investigations, however, the present analysis was extended to include the ultrastructural organization of the brush border zone.

The brush border is composed of minute cylindrical projections from the cytoplasm, which are usually straight and arranged in a perpendicular fashion (Fig. 2, 3, 4, 5, 8). Their apices are gently rounded. For measuring the dimensions of the projections when sectioned parallel to the long axis of the cell, we chose those projections the whole length and width of which could be distinctly discerned. On each projection both the length and the width were determined; the length, measured from the apex of the projection to the level at which the cell border runs parallel with the cell surface between the projections, is $0.91 \pm 0.09 \mu$ (SD=0.35, Tab. 3) and the width is $0.10 \pm 0.003 \mu$ (SD=0.012, Tab. 3). Thus, the length varies considerably among different cells, while the width shows much smaller variations. No correlation between the length and the width could be demonstrated.

From the mean value for the diameter, the cross-section surface of the projection was estimated at $0.008 \mu^2$, while the surface of the whole projection was $0.283 \mu^2$. Thus, the total surface of the projection is about 37 times larger than the cross-section surface.

Determination of the number of projection per surface unit was carried out on cross-cut brush border and a total of $53 \mu^2$ were examined. The mean value was 47 ± 1 projections per μ^2 (SD=10, Tab. 3).

On the basis of these values, the increase of the absorbing surface caused by the projections may be estimated at about 14 times.

A longitudinal fibrillar structure built up by units of very small dimensions may be seen in the projection (Fig. 4, 5). This fibrillar structure may be followed through the whole projection and further, successively decreas-

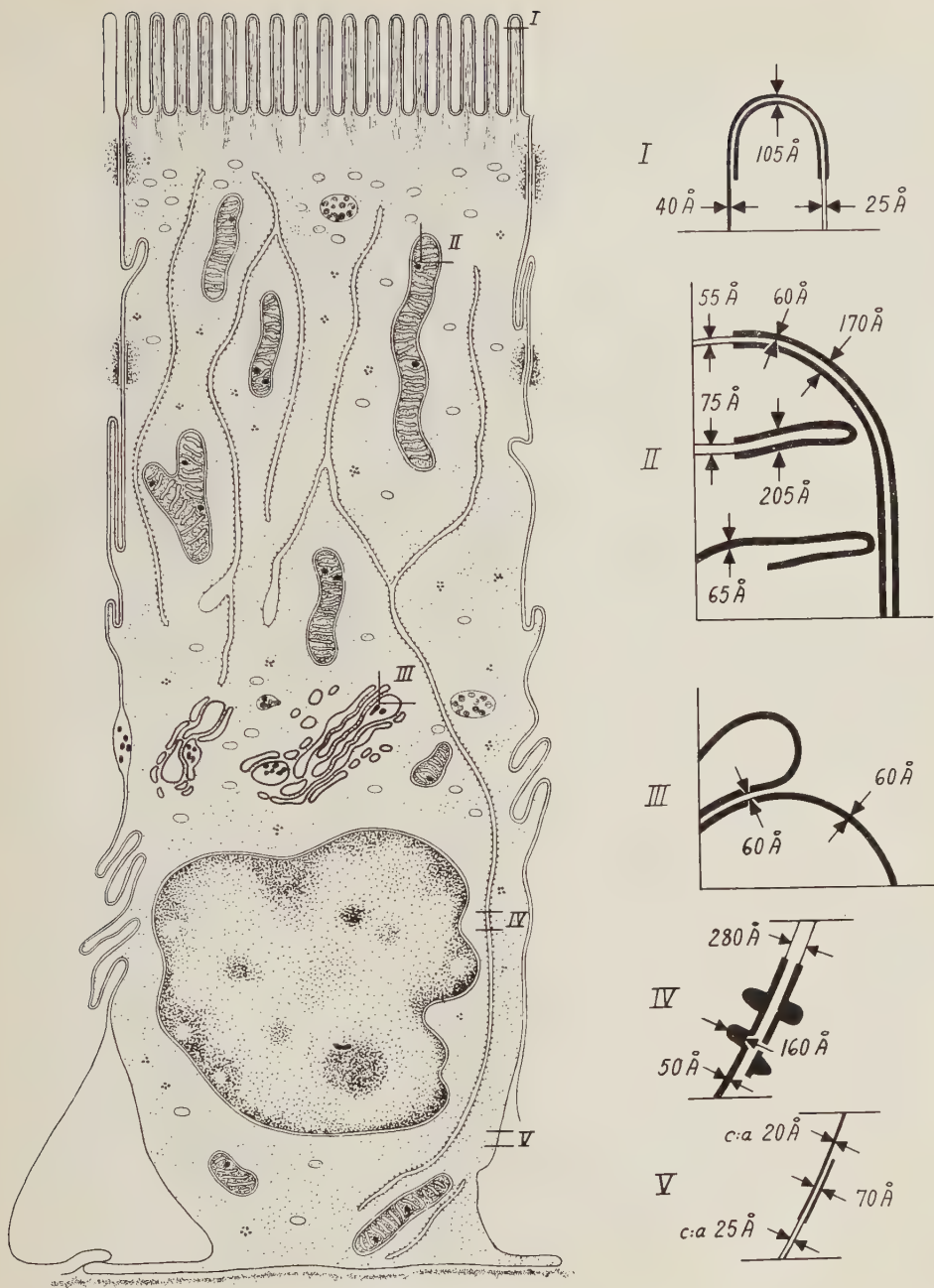


Fig. 1. Schematic drawing of columnar absorbing cell. To the right the dimensions are given for (I) plasma membrane on the free cell surface, (II) mitochondrial membranes, (III) γ -cytomembranes, (IV) α -cytomembranes and (V) plasmamembrane on the cell surface towards the space between the bases of the cells.



Fig. 2. Survey picture of columnar absorbing cells from mouse jejunum with brush border (uppermost), cell boundary (CB), mitochondrion (M), Golgi apparatus (GA) and nucleus (N).

Magnification 6,000 $\times$.

Inset shows light micrograph of the same cell type.

Magnification 1,400 $\times$.

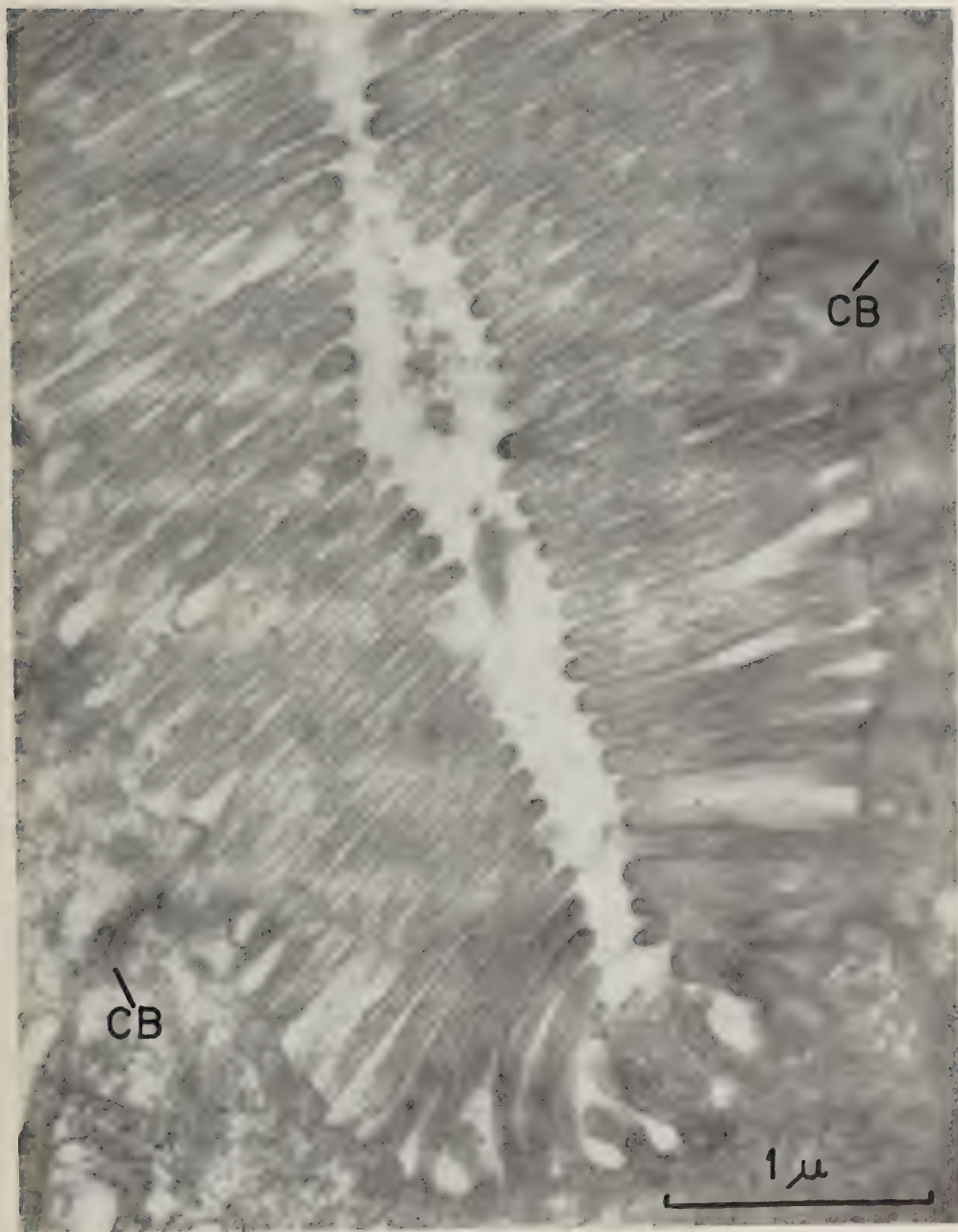


Fig. 3. Survey picture of a pseudocrypt showing brush border projections, cut lengthwise or slightly oblique. CB=cell boundary. Magnification 39,000 $\times$.

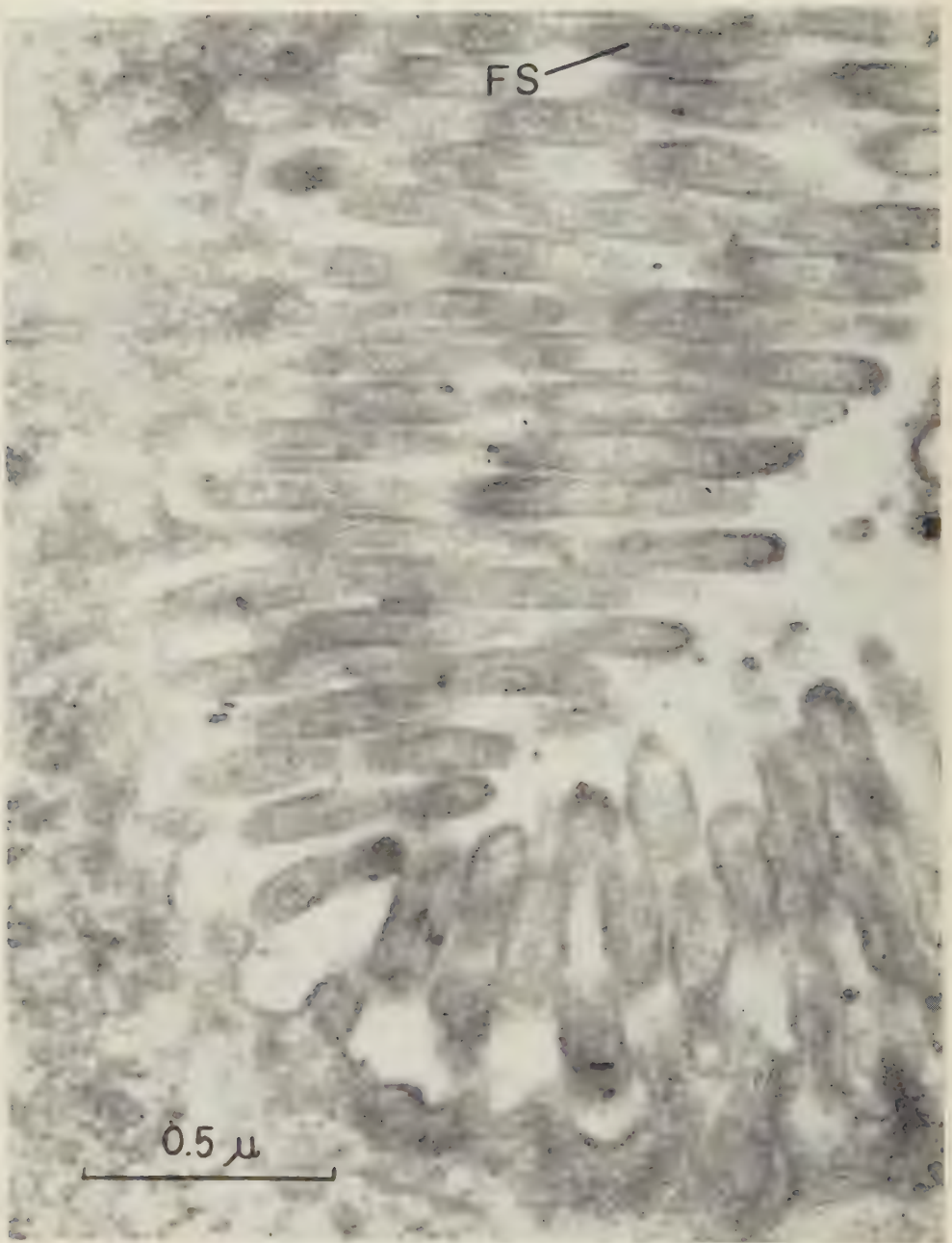


Fig. 4. Slightly oblique section through the brush border zone. The projections are covered by the continuous plasma membrane appearing as two dense layers separated by a less dense space. In the projections is observed a longitudinal fibrillar structure (FS). Magnification 69,000 $\times$.



Fig. 5. Slightly oblique section through brush border projections showing the plasma membrane appearing as two dense layers separated by a less dense space. Magnification 124,000 $\times$.

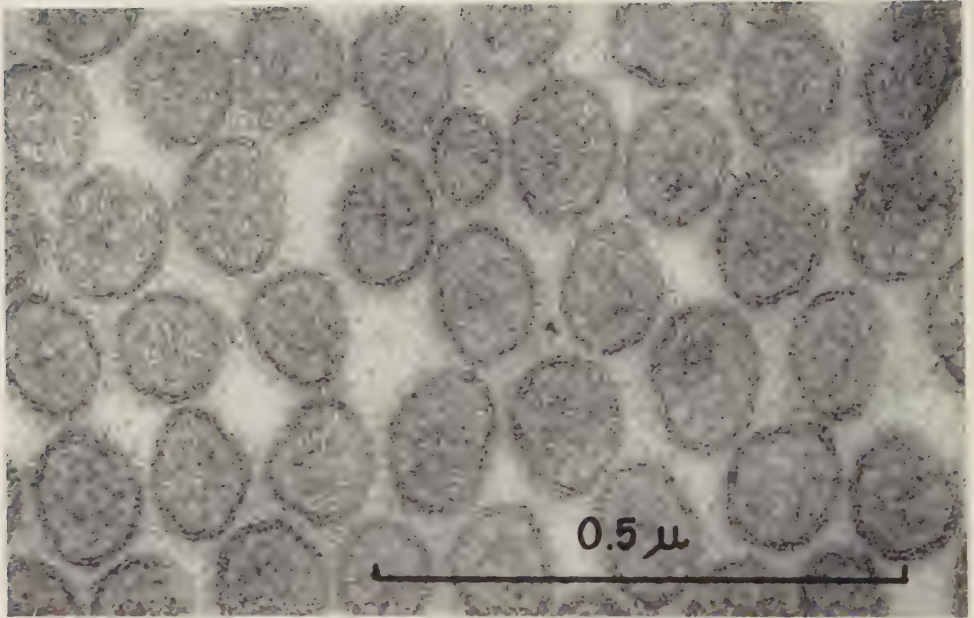


Fig. 6. Transverse section through the brush border zone illustrating the continuous plasma membrane and the innerstructure of the extensions which here is seen as small dots.
Magnification 140,000 $\times$.

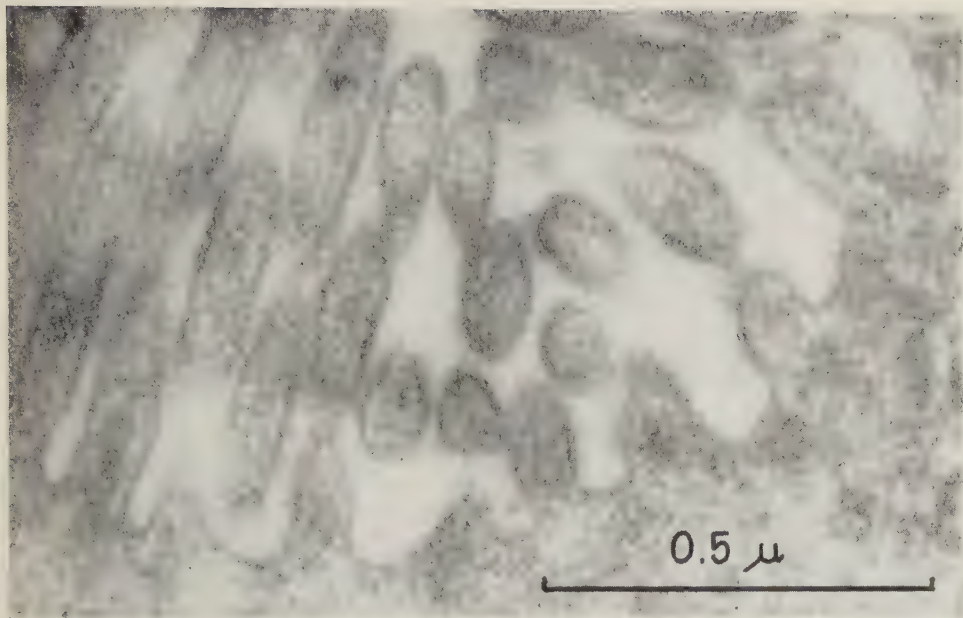


Fig. 7. Slightly oblique section through the basal parts of the brush border extensions showing continuous plasma membrane.
Magnification 103,000 $\times$.

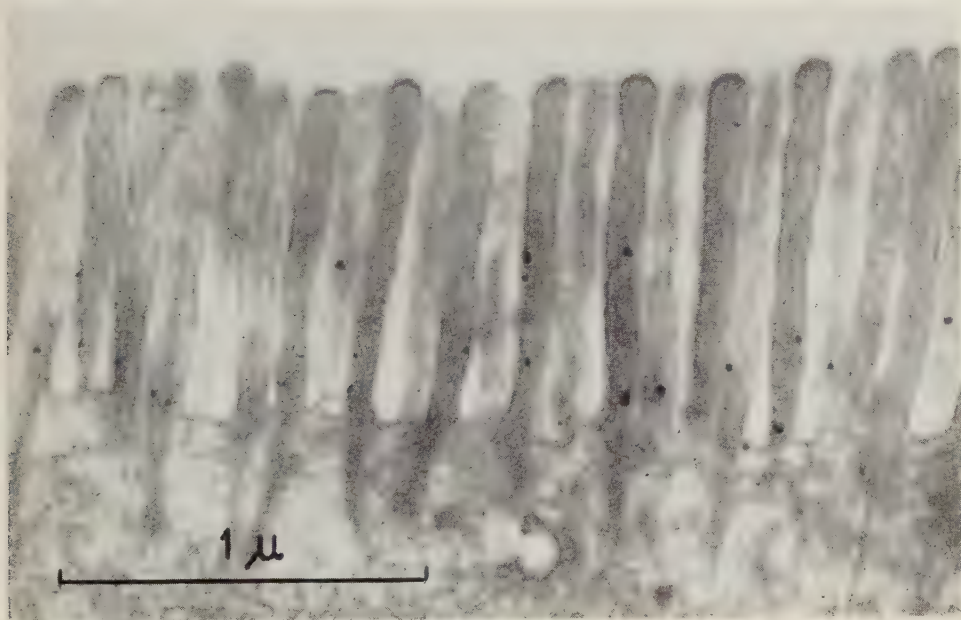


Fig. 8. Longitudinal section through the brush border projections. The fibrillar inner structures confluence and project into the cytoplasm of the cell body as a rootlet.
Magnification 48,000 $\times$.

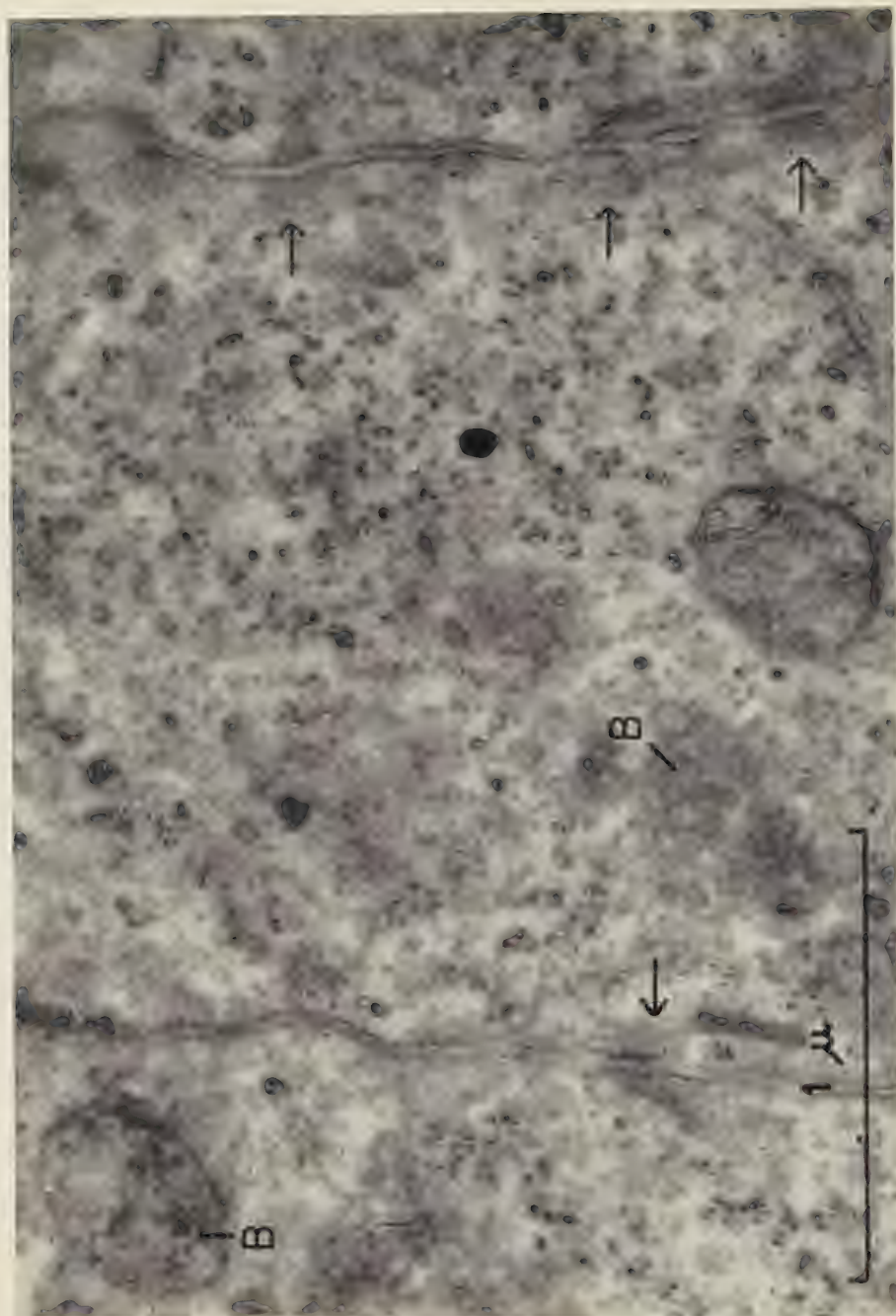


Fig. 3. Longitudinal cell section showing cell boundaries with several horizontal bar regions in which the plasma membrane of each cell appears as two thin layers separated by a less dense space (arrows). Around these special parts of the plasma membranes the cytoplasm has greater opacity. B=vacuole-containing body. Magnification 60,000 $\times$.

ing in width, about $0.5\ \mu$ down into the cytoplasm (Fig. 8). Because of this structure, the cytoplasm in the projections is of greater density than the rest of the cell cytoplasm. The contrast between the opacity of the cytoplasm within the brush border projections and that of the cytoplasm of the cell body is further increased by the fact that the cytoplasm in a zone immediately beneath the brush border contains none of those components typical of other parts of the cell body.

The plasma membrane on the free surface of the cell. The plasma membrane has been studied with particular thoroughness in connection with the brush border projections.

In high-resolution electron micrographs, the plasma membrane appears as an even double-contoured line with two opaque components separated by a less opaque layer (Fig. 4, 5, 6, 7). The total width is $105 \pm 3\ \text{\AA}$ ($SD=8$, Tab. 3) and the distance between the centers of the dark lines is $65 \pm 2\ \text{\AA}$ ($SD=6$, Tab. 3). From these values the dimensions of the individual structures are calculated to be about $40\ \text{\AA}$ for the opaque layers and about $25\ \text{\AA}$ for the intermediate less opaque layer.

The cell membrane extends as a continuous membrane covering each projection and bounding the cell at the bases of the projections without interruption. Thus, in no place does the cell membrane show any pores or breaks, either at the apices of the projections or between their bases (Fig. 7), when examined at a resolution of $30\ \text{\AA}$.

A similar structure of the brush border and cell membrane has been observed in the intestinal epithelium of the rat, the chick, and the frog. In the annelid worm *Stylaria lacustris* the brush border extensions are more widely spaced, of an uneven shape, and exhibit interdigitated cilia, but the cell membrane appears similar, still appearing double-contoured and with the same dimensions (I. SPERBER, personal communication).

Discussion.

The extremely small dimensions of the brush border projections explain why they could not be accurately described until the electron microscopic technique came into use (GRANGER and BAKER, 1950). Earlier described rod structures have in all probability been formations of aggregates of individual projections. This assumption is supported partly by the fact that the width of an extension is below the resolving power of the light microscope, and partly by the information concerning the number of extensions per surface unit given by different authors. BRETTAUER and STEINACH, and others, have sketched 10 and 20 extensions per cell, while the number reported by ZIPKIN is 41.

GRANGER and BAKER assumed that the projections are closely packed and leave only minute spaces open between the units. They calculated about 3,000 projections per cell. We may readily understand this interpretation when studying the brush border in thicker sections cut from base to apex (Fig. 2) or in sections cut at an angle to the longitudinal axis of the cell. An accurate idea of the number of projections can only be obtained from sections cut through the brush border at right angles to the long axis of the projections (Fig. 6). From the determination of the number of projections per surface unit carried out in the present study, it was seen that the cell surface covered by the projections constituted only 35 per cent of the total free surface. This corresponds to 650 to 700 projections per cell, if we, in accordance with GRANGER and BAKER, estimate the free cell surface to be about $15 \mu^2$. Estimates of the free cell surface in the present investigation gave values varying between 10 and $20 \mu^2$.

In both light and electron microscopic studies of intestinal epithelium two lines parallel with the cell surface have been observed; an outer finer line, and an inner (i.e., situated closer to the cell nucleus) broader line. Their nature and relationship to the cell membrane has been much discussed. Electron micrographs taken here show that the outer line appears in thicker sections and represents that part of the cell membrane which runs parallel with the surface of the epithelium between the bases of the projections (Fig. 2). The border granule observed by ZIPKIN, which was said to lie at the level of the outer line, probably is a result of an optical phenomenon caused by the cell membrane in its above-mentioned course. The broader line situated closer to the nucleus represents mainly the basal parts of the inner structure of the extensions which project down into the cytoplasm of the cell body and in thicker sections appear to fuse into a continuous layer.

DANIELLI, in 1936, advanced a theory concerning the structure of the cell membrane which was based on studies of the permeability, surface tension, and electrical resistance of the membrane. The results obtained led to the opinion that the membrane consisted of a lipid layer to which a protein layer was adherent on both sides. The author tried to determine the thickness of this membrane by several indirect methods, working mainly with red cells, and obtained values varying between 33 and $200 \text{ \AA}$. In subsequent communications, DANIELLI stated that in all probability the thickness of the lipid layer in the cell membrane does not exceed $100 \text{ \AA}$ (DANIELLI 1951).

SJÖSTRAND (1953 b) has applied this theory in his interpretation of several membrane structures observed in the cytoplasm. This refers to mitochondria and intracellular cytoplasmic membranes from a number of

different cell types in both freeze-dried and osmium-fixed material. A similar layered structure of the cell membrane, however, could not be observed. The present study shows that in the case of the intestinal epithelium cells a layered pattern similar to that observed in the mitochondrial membranes is present in the cell membrane covering the free surface of the cell. The triple layered cell membrane described by RHODIN (1954) in the proximal tubule cells of the mouse kidney is not comparable to the cell membrane of the free surface of the intestinal epithelial cells. RHODIN measured the thickness of the individual layers to be about 20 Å giving a total thickness of about 60 Å. However, he was able to observe this triple layered structure only in rather restricted regions and it can not be excluded that this layering is due to an arteficial splitting of the membrane. The dimensions of the layers as well as the total thickness of the cell membrane on the free surface of the intestinal epithelial cells are quite different and the triple-layered character is obvious whenever the cell membrane has been sectioned perpendicularly to its surface. Thus, it is obvious that the structure of the cell membrane may vary with the type of cell. As will be seen below the structure of the cell membrane also appears rather different within different regions of the same cell.

The cell membrane covering the free surface of the intestinal epithelial cells has been found to constitute a unit that extends continuously over the whole cell surface without pores or interruptions through which absorption in the form of particles might occur. These investigations, however, do not exclude the existence of discontinuities or pores with a dimension of less than 50 Å.

The spindle-shaped canals observed by J. R. BAKER (1951) in fat absorption are probably the spaces which occur between the projections and which, owing to accumulation of fat, have become sufficiently wide to be seen in the light microscope.

THE PLASMA MEMBRANE ON THE CELL SURFACES FACING ADJACENT CELLS AND THE BASEMENT MEMBRANE.

Earlier observations.

Light microscopic studies have failed to demonstrate whether the cells in a tissue are separated from one another by a true cell membrane or simply by a denser zone of the cytoplasm, a so-called ectoplasmic layer.

Electron microscopic investigations of osmium-fixed material from different tissues have shown that the cells are delimited by a distinct opaque

membrane. In the retinal rods (SJÖSTRAND 1953 c), the proximal tubule cells of the kidney (SJÖSTRAND and RHODIN 1953, RHODIN 1954), and the secretory cells of the pancreas (SJÖSTRAND and HANZON 1954 a), this opaque membrane was about 60 Å in thickness. In places where two cells lay close together the opaque membranes were separated by a layer of low density and constant width. In the above-mentioned cell types, the width of this less dense intermediate layer was about 120 Å. In the tubular cells of the kidney the cell membrane was described as consisting of three components, two opaque layers surrounding a less opaque layer, each layer being about 20 Å in width (RHODIN 1954). A specialized region of the cell membrane was demonstrated by SJÖSTRAND (1953 c) in the retinal rods of the guinea-pig eye at the level where the external limiting membrane, as described in light microscope studies, should be located. At this level the cytoplasm at the plasma membrane was especially opaque. The opaque regions formed ring-shaped peripheral zones, which were interpreted as representing an intracellular skeleton. A similar structural organization has also been observed in the epidermis of the frog skin (OTTOSSON, et al., 1953), the intercalated discs of the heart muscle in frog and guinea-pig (SJÖSTRAND and ANDERSSON 1954), and in the proximal tubule cells of the mouse kidney (RHODIN 1954).

Results.

In those areas where two cells lie close together the two cell membranes of adjacent cells appear as two opaque lines separated by a less opaque layer of fairly constant width (Fig. 9, 10). To determine the dimensions of these three components, the total width and the distance between the centers of the opaque lines were measured, and the mean values 250 ± 8 Å (SD=28, Tab. 3) and 180 ± 6 Å (SD=22, Tab. 3), respectively, were obtained. From these values the width of the opaque lines was estimated at about 70 Å, while the interspace was about 100 Å. Analysis of the cell membrane in its course along the basement membrane and on the surfaces facing the space frequently present between the basal parts of the cells shows that the membrane in these areas is built up of three components each being 20 to 25 Å in width, two of them being opaque and separated by a third, less opaque layer (Fig. 13). As regards those parts of the cell membrane which make contact with adjacent cells, only a few observations which demonstrate a similar layered structure in these areas have been made, and these observations concern only very short distances.

Specialized regions of the plasma membrane are observed in some areas, mainly in close relation to the free cell surface. Thus, at the transition between this surface, where it consists of two opaque layers separated by a less opaque layer (zone I, Fig. 11), and the surfaces which are in contact

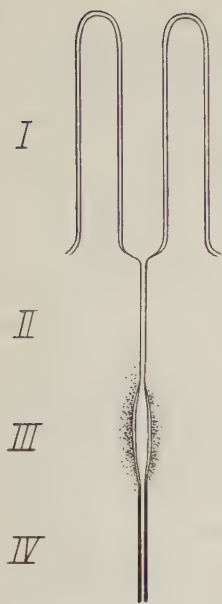


Fig. 11.

Schematic drawing of some parts of the plasma membrane. Explanation in text.

with adjacent cells, the plasma membrane changes into a membrane with a single opaque layer about 50 Å in width (zone II, Fig. 11). The distance between these layers from adjacent cells is about 100 Å. This condition is retained for a distance of $\frac{1}{4}$ to $\frac{1}{2}$ μ from the brush border region. Further below the opaque layer, the cell membrane appears split into two opaque layers separated by a less opaque space (zone III, Fig. 11) (Fig. 9, 12). Each of these layers is about 50 Å in width. Therefore, within this region, which reaches another $\frac{1}{2}$ μ in basal direction, there are four opaque layers arranged in pairs separating the cytoplasm of two adjacent cells. The space between the two pairs in this region seems to be slightly widened, to approximately 200 Å, but has the same opacity as in other parts of its course. Close to this part of the plasma membrane the ground substance of the cytoplasm shows an increased opacity which is most marked nearest the membrane (Fig. 9, 10, 12). The width of this zone is about $\frac{1}{4}$ μ . Zone IV at Fig. 11 shows the appearance of the membrane in the part of its course where the membranes from adjacent pairs run close together.

Analogously built up zones are occasionally observed in several places along the two contact-making membranes from adjacent cells (Fig. 9), but have never been seen in those parts of the cell membrane which do not make contact with adjacent cells.

The cell surfaces which are in contact show abundant interdigitations of varying shape and size. These appear to be most numerous in the basal parts of the cells (Fig. 14).

As has been mentioned above, the space of low opacity situated between the two opaque layers of the membranes in contact is of fairly constant width over the larger part of its course. In its lower half, however, small elongated widenings are often observed, which in some instances contain round bodies of varying size and density. In the region between the nuclei and the basement membrane, this space is usually widened to form larger intercellular spaces which in the sections appear as triangular formations with the acute angle directed upwards. At the base, these spaces are separated from the basement membrane by the widened basal parts of the cells which appear in sections as foot-like projections, which are in contact with the basement membrane (Fig. 16). The size of these triangular widened

spaces varies considerably, probably depending on the functional state of the cells. Thus, during absorption they sometimes assume a considerable size, the apex reaching up to more than half the length of the cells and the width exceeding that of the surrounding cells (ZETTERQVIST 1954). In these cases too, however, the foot-like projections on the basement membrane make contact with each other.

These basal distensions of the intercellular space are often found to contain white blood cells (Fig. 2, 14).

Discussion.

On the surfaces facing adjacent cells, the cell membrane of the intestinal epithelial cell consists of one opaque component, which has the same dimension as described for the membranes covering the retinal rods and the secretory cells of the pancreas. In the parts facing the basement membrane and the widened spaces which often occur between the basal parts of the cells, a triple-layered structure of the cell membrane is observed in which the single components have the same dimensions as is described for the cell membrane of the proximal tubule cells of the kidney (RHODIN 1954). In those parts of the membrane which are in close contact with adjacent cells, on the other hand, this layered structure cannot be seen.

The fold-like structures which occur along the whole surface where the cell makes contact with neighboring cells are interdigitations which may serve to strengthen the connections between the cells, as has earlier been supposed in the case of, for instance, the liver cells (FAWCETT 1955). It is also probable that they constitute a reserve which makes possible a considerable increase of the cell volume in absorption, or to make possible the formation of the intercellular spaces, the form of which means a large surface area for the bounding cells.

The areas of the cell membranes which are in contact with a dense cytoplasmic zone seem to correspond to the "Schlussleisten" or "terminal bars" known from light microscopic studies (ZIMMERMANN 1898, and others). Their function has been assumed to be that of closing the intercellular space at the end near the lumen. As has been suggested by SJÖSTRAND (1953 c) and SJÖSTRAND and ANDERSSON (1954) it seems probable also that these structures may constitute places where an especially firm contact is established between the cells. It is interesting to note that in some cases two or three such structures are observed around the same intercellular space at different distances from the free cell surface. This observation does not seem to have been made by the light microscopists.

Regarding the less opaque space of uniform size that is observed between the opaque layers of adjacent cell membranes, it has been assumed (Sjö-

STRAND 1956 a) to correspond to an organized layer of material, for instance, lipids. It has been suggested that this lipid layer constitutes a part of the plasma membrane. Thus, the total width of this membrane should be about 120 Å.

BASEMENT MEMBRANE.

Earlier observations.

Light microscopic studies have demonstrated the presence of a so-called basement membrane between the cells in an epithelium and the subjacent connective tissue, but have failed to provide any reliable data concerning the dimensions and structure of this membrane and its relationship to adjacent tissues. With such a technique, however, the whole layer between the epithelium and the cells in the subjacent tissue seems to have been interpreted as basement membrane.

Using the electron microscope, the basement membrane associated with a number of epithelia has now been analyzed. It has, in most instances, appeared to consist of a structureless membrane with a relatively low opacity, which is separated from the cell membrane by a light space. Its width seems to vary from cell type to cell type and has in the epidermis of frogs been estimated at 200 to 300 Å (OTTOSSON, et al., 1953), in the proximal tubule cells of the mouse kidney at 800 Å (RHODIN 1954), in the secretory cells of the mouse pancreas at 150 to 400 Å (SJÖSTRAND and HANZON 1954 a), in corneal epithelium from rats at 300 Å (JAKUS 1954), and from mice at 600 Å (SHELDON 1956). In the basement membrane associated with the last-mentioned epithelium, JAKUS has described a condensation of fibrils which are considerably narrower in width than are the collagen fibrils of the stroma, while SHELDON has not been able to observe any such fibrillar structure either in preparations fixed in osmium tetroxide in the usual way or in specimens treated with phosphotungstic acid. In the capillary wall of the glomerulus, RHODIN (1955) described a basement membrane 650 Å in thickness, in which there was an indication of a lamellated structure giving the impression of a spongy system in the layer. The basement membrane in association with the "Flaschenzellen" occurring in kidney of the xenopus-frog has by BARGMANN, et al., (1955) been estimated to be about 500 Å in diameter. These authors observed a regular delicate cross-striation in the basement membrane. An interpretation of the basement membrane, which seems to correspond to the light microscopic description, was presented by WEISS and FERRIS (1954 a, b) in an analysis of larval amphian epidermis. They described this membrane as being built up of about 20 layers of straight collagen fibers stacked cross-wise and filling the areas between the epidermis and the subjacent

mesenchymal cells. A membrane probably corresponding to the one designated as the basement membrane by other electron microscopists was described by these authors as a "rather hyalin membrane", 600 Å in thickness, between the collagen layer and the epithelium. This membrane was reported to contain a single layer of dense granules.

Results.

The basement membrane which is found in association with the basal surfaces of the intestinal epithelial cells appears as a continuous, vaguely defined, membrane, the opacity of which is lower than that of the adjacent cell membrane (Fig. 15, 16). Its width is 270 ± 15 Å (SD=49, Tab. 3). Between the basement membrane and the opaque layer of the cell membrane is seen a layer of low opacity, the width of which usually is less than that of the basement membrane but occasionally more, depending on irregularities in the surface of the epithelial cells. No inner structure in the basement membrane has been observed. It is interesting to note that the basement membrane, which delimits the endothelial cells of capillaries, has a close contact with the adjacent cells, contrary to the above-mentioned conditions (Fig. 15).

Irregularly occurring projections of varying shape and size were seen leading from the surface of the membrane towards both the epithelium and the subjacent tissue.

Discussion.

In this, as in the majority of the earlier electron microscopic studies, it has been considered most suitable to apply the term basement membrane to the membrane readily discernible in thin sections, which is found in immediate association with the epithelium, and not to include adjacent collagen or other tissue in this term.

With respect to the finer structure of this membrane, an inner structure with a longitudinal orientation analogous to the one described by JAKUS (1954) and RHODIN (1955) has not been observed. Nor has it been possible to distinguish any cross-striation as reported by BARGMANN, et al., (1955) in the basement membrane associated with the "Flaschenzellen" from the frog kidney.

Regarding the distance between the epithelial cell and the basement membrane, it is possibly a post-mortem effect. However, this does not seem to be the case as no increase of the distance can be observed in specimens fixed 40 minutes after death, neither between the epithelial cells and the basement membrane nor between the capillary endothelial cells and their basement membrane.

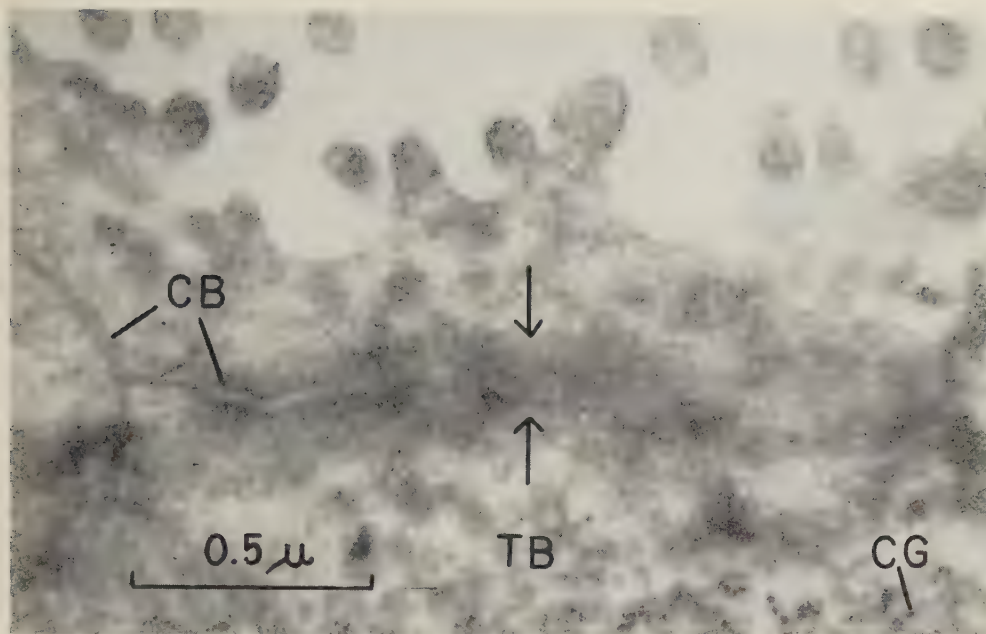


Fig. 10. Longitudinal section through a terminal bar region (TB) showing the pronounced opacity around the cell boundary (CB). CG=cytoplasmic granules. Magnification 63,000 $\times$.

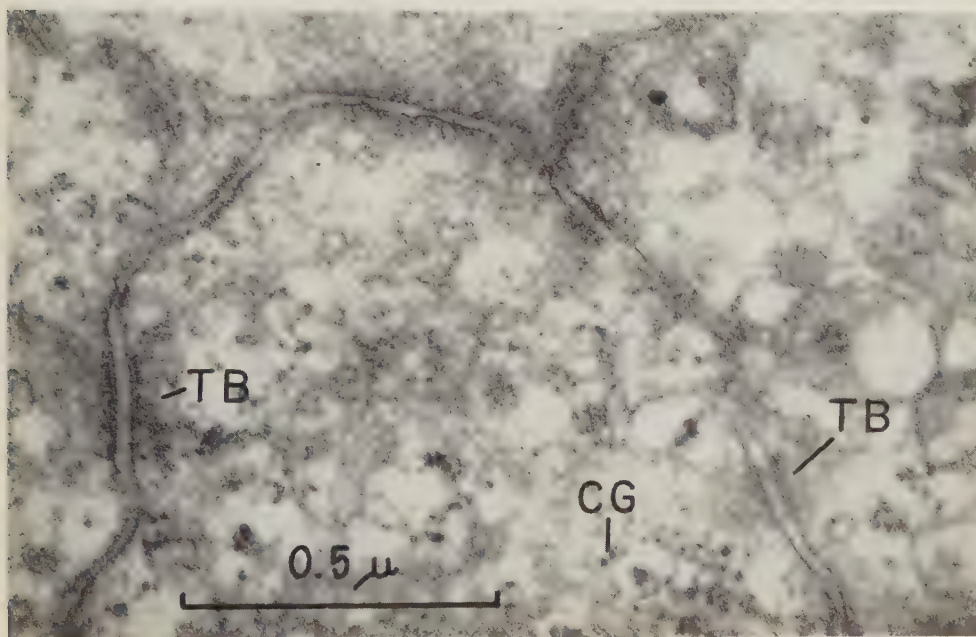


Fig. 12. Section through terminal bar regions (TB) showing double-contoured plasma membranes and adjacent opaque cytoplasm. CG=cytoplasmic granules. Magnification 79,000 $\times$.

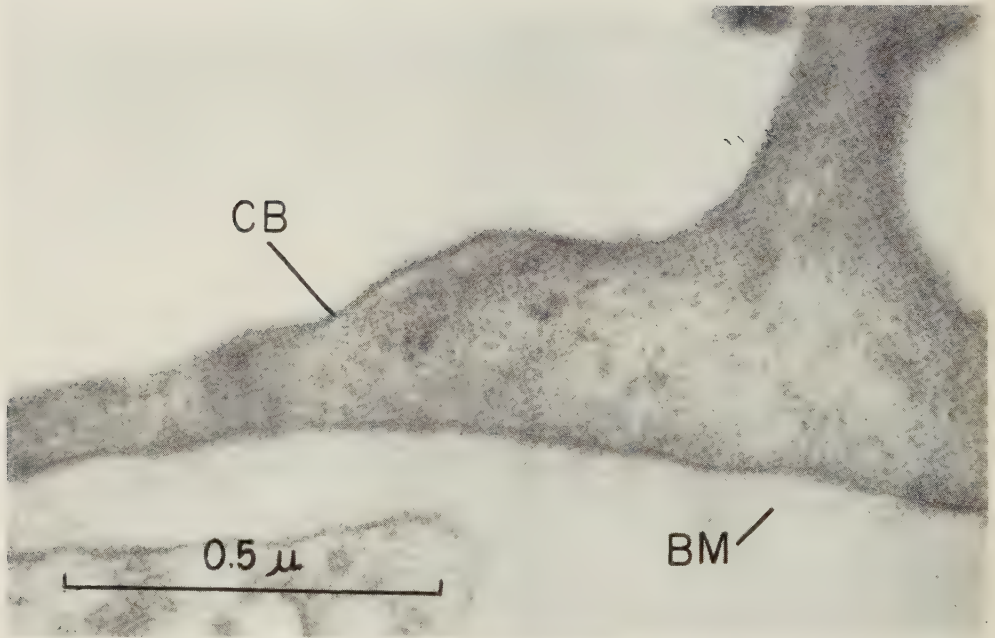


Fig. 13. Section through a foot-like cell projection adjacent to the basement membrane (BM). The plasma membrane (CB) appears as two dense layers separated by a less dense space.

Magnification 102,000 $\times$.

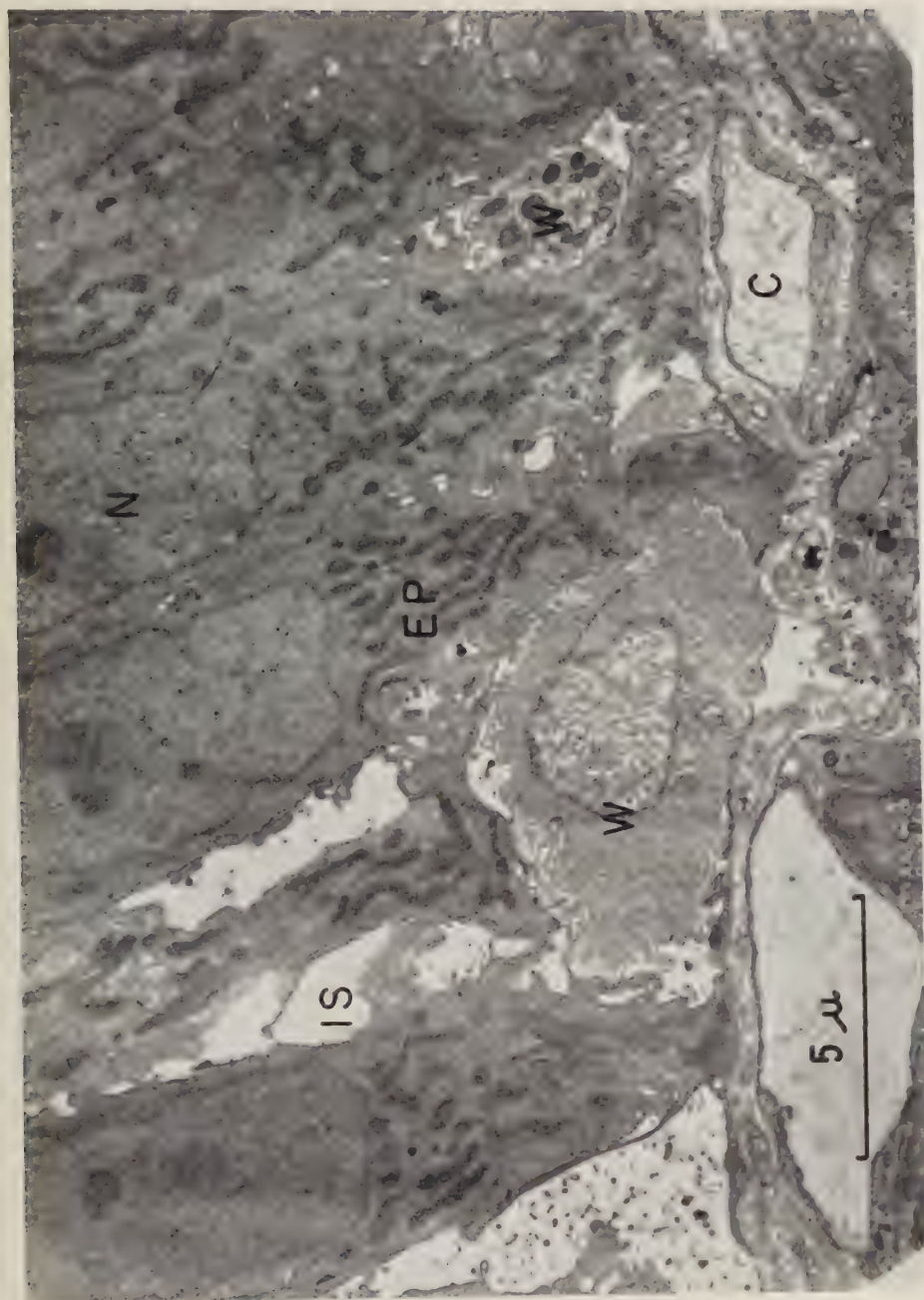


Fig. 14. Survey picture of basal parts of epithelial cells (EP) showing intercellular spaces (IS) which often contain white blood cells (W). The white blood cell to the left seems just to have passed the basement membrane. N=nucleus. C=capillary in subepithelial tissue. Magnification 7,000 $\times$.

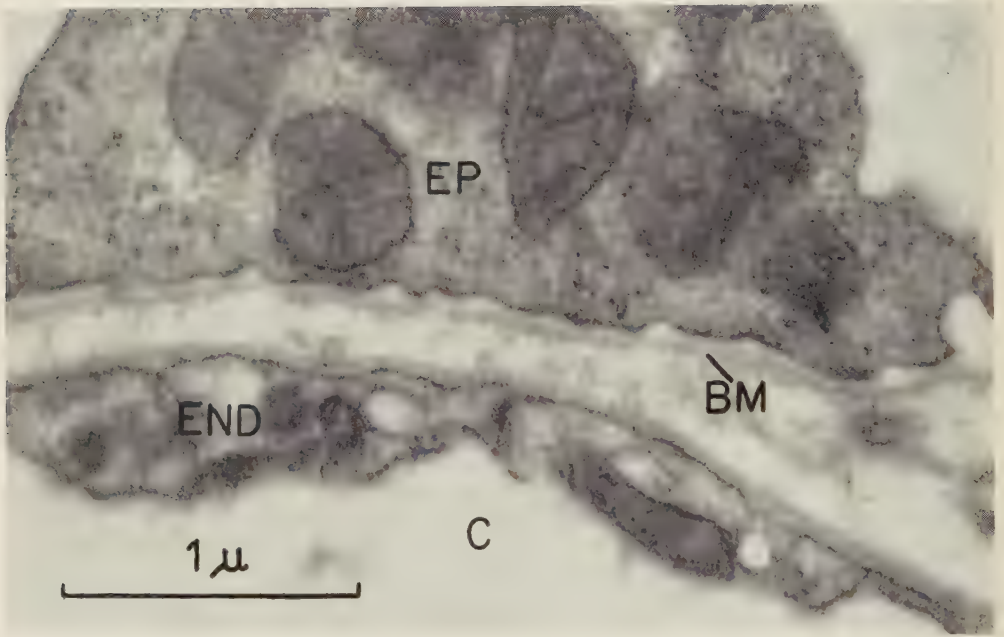


Fig. 15. Section through epithelial cell (EP), basement membrane (BM) and endothelial cell (END) of a capillary (C). Between the epithelial cell and the basement membrane is observed a space, while there is no space between the capillary endothelium and its basement membrane.
Magnification 39,000 $\times$.

MITOCHONDRIA.

Earlier observations.

ALTMANN (1890) seems to have been the first to present a detailed description of those ubiquitous cell components which we have come to call mitochondria after the suggestion of BENDA (1900). However, as COWDRY points out (1932), it is probable that the cytoplasmic structures described by FLEMMING, KÖLLIKER, HELD, and others, may well have been mitochondria.

Mitochondria can be seen with phase contrast microscopy in living cells as round or rod-shaped structures which undergo continuous changes in shape and continuous displacements in the cytoplasm. Their shape, size and number vary from cell type to cell type, but are apparently constant in cells with similar function.

At first, electron microscopic observations were carried out on mitochondria which had been isolated by differential centrifugation (CLAUDE and FULLAM 1945, DALTON, et al., 1949). By this technique membrane structures and round bodies could be isolated, the size of which varied with the suspending medium (DALTON, et al., 1949).

A more complete knowledge of mitochondrial structure was obtained after the development of sectioning methods. By such methods which produced ultrathin sections, permitting a resolution of better than 30 Å, SJÖSTRAND¹ (1953 b, c) was able to show that the mitochondria in the retinal rods of the guinea-pig eye, the secretory cells of the mouse pancreas and — in cooperation with RHODIN (1953) — the proximal tubule cells of the mouse kidney, were surrounded by a double-contoured membrane and possessed inner membranes which were mainly transversely oriented and also double-contoured. The thickness of the inner and outer double-contoured membranes of the mitochondria was said to vary between 150 and 170 Å in different cell types, the opaque components being 45—60 Å and the interspace 40—70 Å. The distance between the inner membranes was different in mitochondria from different organs. Dark areas 200—700 Å in diameter were observed between the inner membranes in the mitochondria from the proximal tubule cells of the kidney.

SJÖSTRAND interpreted the structure of the mitochondrial membranes in the light of DANIELLI's postulation that a cell membrane is constructed of a bimolecular lipid layer sandwiched between two protein layers and assumed that the observed double membranes of the mitochondria were analogously organized.

Independent of these studies PALADE (1952 b) investigated the structure

¹ Presented at the Scand. El. Micr. Soc. Meeting in Upsala, June 1952 and at the EMSA Meeting, November 1952.

of mitochondria from different cell types and presented an opinion that they were bounded by a single layered membrane 7—12 $m\mu$ in width, and contained inner membranes arranged perpendicularly to the long axis. These inner membranes were interpreted as folds from the outer membrane which, as a rule, did not reach the center of the mitochondrion, where, thus, a central canal or cavity was formed. The folds were described as being made up of a central layer of relatively light material surrounded by two denser layers. The ground substance of the mitochondria appeared homogenous. In some cell types, e. g., epithelial cells of the intestine and kidney, granules the size of 200—300 Å were observed between the membrane folds.

After further analysis, PALADE (1953 a) confirmed that the membrane surrounding the mitochondrion appeared to be double, but stated that the transverse membranes were folds of the inner layer of the outer mitochondrial membrane.

RHODIN (1954), after studying the proximal tubule cells of the mouse kidney, was able to confirm the description by SJÖSTRAND and RHODIN (1953) of the organization of the mitochondria. The internal membranes were described as extending straight across the diameter of the mitochondrion, a segment of their circumference being in contact with the inner aspect of the outer membrane. The distance between the double-contoured internal membranes was estimated at 200 to 400 Å. The dark layers in the double membranes might appear split into three layers. The opaque areas which were found in places between the inner membranes, consisted of accumulations of round or oval granules, 75 to 150 Å in diameter. The ground substance in the mitochondria was finely granular and the dimensions of the granules was estimated at 40 to 50 Å.

SJÖSTRAND and HANZON (1954 a) further analyzed the secretory cells of the mouse pancreas. They found that the outer membrane was triple-layered, thus confirming their earlier observations, and that the double-contoured inner membranes oriented perpendicular to the long axis made contact with the outer membrane within a segment of their circumference. The internal membranes formed only incomplete septa. No central space free from inner membranes could be seen. With a few exceptions, no continuity between the central space in the inner and the outer membranes was observed. Between the inner membranes, opaque spherical bodies with a diameter of 180 to 400 Å were also seen.

In light microscopic studies of the absorbing cells of the intestinal epithelium the mitochondria have been described as rod-shaped in the parts of the cell located between the nucleus and the brush border, while those observed in the basal parts of the cytoplasm have been described as round.

DALTON (1951 b), however, after electron microscope examination of the basal parts of the cytoplasm, found that filamentous mitochondria occurred in great numbers also in these parts.

Results.

The mitochondria are, on the whole, uniformly distributed in the cells except in the finely granular zone nearest the brush border, where they are never found.

These organelles in the intestinal epithelial cells are usually rod-shaped, but round and more or less irregularly shaped specimens also occur (Fig. 17, 18). The rod-shaped bodies, however, predominate in both the supra-nuclear and basal parts of the cell. In the area above the nucleus they are primarily oriented parallel to the long axis of the cell, while those occurring basally are more irregularly arranged. The length of the mitochondria varies considerably, the longest sectioned mitochondrion measuring about 3 μ . However, since the investigation was carried out on ultrathin sections and since the mitochondria often show an irregular wavy course, it is probable that this value does not represent the maximum length. The width was found to be fairly constant with a mean value of $0.28 \pm 0.002 \mu$ ($SD=0.029$, Tab. 4).

As in previously examined cell types, the mitochondria in the intestinal epithelial cells have a well-defined ultrastructure. Thus, they possess a double-contoured limiting membrane consisting of two opaque layers defining a less opaque space, and internal membranes which are also double-contoured and, as a rule, arranged perpendicular to the long axis.

The two opaque layers of the outer membrane, are separated by a rather constant distance. The total thickness of the outer membrane is $170 \pm 5 \text{ \AA}$ ($SD=28$, Tab. 5) and the distance between the centers of the opaque lines $110 \pm 4 \text{ \AA}$ ($SD=20$, Tab. 5). From these values the thickness of the opaque layers is calculated to be about 60 $\text{\AA}$ and the width of the intermediate layers to be about 55 $\text{\AA}$.

The membranes found within the mitochondria show the same structure as the outer membrane. They differ from the outer membrane, however, in that the light intermediate layer is on an average slightly wider and, moreover, shows greater variations in thickness. The total width of the inner double-contoured membrane is $210 \pm 6 \text{ \AA}$ ($SD=36$, Tab. 5) and the distance between the centers of the opaque lines $140 \pm 4 \text{ \AA}$ ($SD=25$, Tab. 6). The calculated values for the thickness of the opaque component are about 65 $\text{\AA}$ and for the intermediate layer about 75 $\text{\AA}$.

Statistical analysis of the values obtained from measuring outer and inner membranes from 17 mitochondria (from 17 cells and 14 animals)

show that the difference in thickness between outer and inner membranes is highly significant.

In sections, the inner double-contoured membranes may extend across the entire diameter of the mitochondrion or only a part of that distance. Contact between the inner and outer membranes seems in most cases to be established only through the opaque components, the lighter intermediate layer in the internal membranes being enclosed by the fusion of the two opaque layers at the ends of the membrane. Sometimes, however, a direct continuity is observed between the light intermediate layers in inner and outer double-contoured membranes.

Generally, the inner membranes are parallel to each other, although the distance between the membranes varies between 200 and 600 Å.

Somewhat rounded, opaque bodies with a diameter of 200 to 600 Å are observed scattered between the inner double-contoured membranes (Fig. 18, 23). The membranes often show a curved course around these bodies.

The ground substance in the mitochondria is of greater opacity than that of the surrounding cytoplasm. Despite the high resolution achieved in the electron micrographs in the present study, it cannot be determined whether this ground substance is finely granular or homogeneous.

Discussion.

Light microscopic studies of the mitochondria of the intestinal epithelium have led to the opinion that they are rod-shaped in the apical parts of the cell and mostly rounded in the basal cytoplasm. This has not been confirmed by electron microscopic studies, since it has been demonstrated that the basal mitochondria, too, are mostly rod-shaped, as pointed out earlier by DALTON (1951 b). A probable explanation for this conclusion is that the mitochondria above the nucleus are primarily oriented parallel to the long axis of the cell, while those occurring in the basal cytoplasm are more irregularly arranged. Hence, a longitudinal section through the cell has proportionally a greater number of cross-cut mitochondria in the areas basal to the nucleus than distal to the nucleus.

The ultrastructural organization of the mitochondria in these cells is principally the same as in the proximal tubule cells of the mouse kidney, the retinal rods of the guinea-pig eye, and the secretory cells of the mouse pancreas — as they have been described by SJÖSTRAND and his associates — with a double-contoured outer membrane and internal membranes also double-contoured and oriented transversely to the long axis of the organelle.

Further analysis, however, shows that certain differences exist, e.g., in the dimensions of the outer and the inner membranes, in the shape and extension of the latter and in their relationship. These variations are suf-

ficiently distinct to give to each of these types of mitochondria a characteristic appearance.

With regard to their shape and extension, the inner membranes in the mitochondria from the intestinal epithelial cells seem to have the shape of plates or lamellae with an outline that is sometimes even and circular but more often irregular, like the pieces of a jigsaw puzzle. Thus, their shape corresponds rather well to the shape of the mitochondria from the inner segment of the guinea-pig eye retinal rods as described by SJÖSTRAND (1953 c). The contact established between outer and inner membranes, when the latter are of an irregular shape, will occur in several places along the circumference of the lamella. On closer examination of these points of contact, it appears probable that they are maintained, as a rule, by the opaque components from the respective inner and outer membranes, even if occasionally it is found that the less opaque layers from the inner and outer membranes occurring between the opaque components also make contact with each other.

This investigation has not provided any evidence supporting the view advanced by PALADE 1952 b, 1953 a) that the inner membranes are folds projecting from the outer membrane and making contact with it all along their base. Nor has the investigation shown the presence of a central canal in preparations fixed in the usual way. A mitochondrial structure highly reminiscent of PALADE's picture is observed in our preparations in which post-mortem changes have been provoked.

THE GOLGI APPARATUS.

Earlier observations.

Ever since CAMILLO GOLGI in 1898 described the "apparato reticulare interno" in nerve cells, much research has been conducted on this cellular component and a great number of studies on its morphology has been published.

The appearance of this organelle, which varies from cell to cell and according to the method of fixation, has given rise to several interpretations of its structure. A fibrous network, a fenestrated lamella, vacuoles of varying shape and size, anastomosing canals, a liquid or semi-firm mass, a homogeneous substance, are some of the descriptions of the structure of the Golgi apparatus.

This variety of interpretations has led to the opinion that a Golgi apparatus *per se* does not exist, and that the described structures are artefacts produced by fixation, e.g., myelin figures (PALADE and CLAUDE 1949), or that they are other cell elements which have been interpreted erroneously

as being the Golgi apparatus, e.g., lipid droplets (J. R. BAKER 1949). This opinion is also favoured by those workers who have failed to demonstrate in light microscopic studies of living cells a special component in those areas of the cell in which the Golgi apparatus is said to occur.

Reviews of the literature on light microscopic research have been presented by KIRKMAN and SEVERINGHAUS (1938 a, b, c), HIRSCH (1939), HIBBARD (1945), BOURNE (1951), BENSLEY (1951), DALTON and FELIX (1953, 1954), and others.

The first electron microscopic studies of the Golgi apparatus, which were carried out at a low resolution, showed an unspecific structural arrangement recalling the light microscopic picture. DALTON (1951 c, 1952), after studying epithelial cells from mouse intestine and liver, demonstrated that the Golgi apparatus was made up of "strands" of osmiophilic material. In the intestinal epithelial cells, in close association with these strands, he also observed vacuoles of varying size, larger in the cells from the villi and smaller in the cells of the intestinal crypts. Further studies of epithelial cells of the epididymis and the duodenum confirmed these observations. Comparative light microscopic, phase contrast microscopic and electron microscopic analyses (DALTON and FELIX 1953) showed that the Golgi apparatus in these cell types is a distinct cell unit, and that the droplets, supravitaly stained with methylene blue or neutral red, which were seen near the Golgi apparatus had no morphologic relationship with this cell organelle nor were converted into it by the action of the fixing chemicals.

The results of further analyses of the Golgi apparatus were published by several authors in 1954.

SJÖSTRAND and HANZON (1954 b, c) studied the ultrastructure of secretory cells in the mouse pancreas at a resolution better than 30 Å. They found that the Golgi apparatus was divided into several zones in every cell, each one containing granules and tightly packed membranes arranged in pairs, with two to five pairs per zone. In places, the membrane pairs were separated by vacuoles. These structures were embedded in a homogeneous granulated or reticulated ground substance in which the α -cytomembranes typical of other parts of the cytoplasm did not appear. The thickness of the membranes was about 60 Å and the width of the space between them also about 60 Å. It was pointed out that these membranes were not comparable to the earlier described cytoplasmic double membranes. Membranes of the type occurring in the Golgi apparatus are called γ -cytomembranes by SJÖSTRAND (1956 b). In the Golgi zone granules were observed constituting a constant component in this type of cell. The size of the granules appearing in the Golgi zone varied from 40 Å up to 0.5 μ , the latter value being

the dimension of zymogen granules. An intimate topographic relationship between membranes, Golgi granules, and zymogen granules was demonstrated.

RHODIN (1954) analyzed the proximal tubule cells of the mouse kidney under the same conditions as those used by SJÖSTRAND and HANZON, and found that the Golgi apparatus in this region was made up of double membranes, the thickness of the single dense layer being about 60 Å, and the distance between them about 90 Å. The width of the space between the double membranes ranged from 50 to 200 Å in those places where it was not widened into vacuolar formations. At the periphery of the Golgi apparatus vacuolar formations were observed with a diameter of 400 to 600 Å, defined by a single membrane 60 Å in thickness.

In parallel electron microscopic studies of the Golgi apparatus in the epithelial cells of the epididymis, DALTON and FELIX (1954) found that it was composed of large vacuoles, lamellae and granules. The vacuoles appeared in cross-section to be arranged in the shape of a horse-shoe and were surrounded by concentrically arranged lamellae. These lamellae were said to be comparable to the "double structures" described in the cytoplasm of the secretory cells of the pancreas and other organs, although those appearing in the Golgi apparatus had no attached granules. The width of the osmiophilic layers in the lamella was estimated at about 70 Å and the width of the intervening layer also at about 70 Å. The space between the lamellae was about 140 Å in thickness. The granules regularly occurring in the Golgi zone had a diameter of about 400 Å.

A similar arrangement of the Golgi apparatus is reported by FAWCETT (1955) in liver cells and by HAGUENAU and BERNHARD (1955) in different cell types. PALAY and PALADE (1955), who investigated the conditions in the neuron, described the structure as an agranular reticulum and stated that its configuration was analogous to "endoplasmic reticulum". They claimed that they could identify intermediate forms between these two structures.

Results.

In the columnar absorbing cells of the intestinal epithelium, the Golgi apparatus is found in close relation to the distal pole of the nucleus (Fig. 2), where it constitutes a well-defined structure which is different from the rest of the cytoplasmic components and usually is made up of several small units between which there seems to be no communication. These groups extend mainly in a plane perpendicular to the long axis of the cell, but there are also extensions in a proximal and distal direction. No Golgi elements, however, are found in the distal half of the area between the nucleus and the brush border (Fig. 2).

The Golgi apparatus is made up of the following components: a ground substance, membranes arranged in pairs in which the two single membranes are often separated by vacuolar spaces of varying dimensions, and small vacuoles or vesicles.

The Golgi ground substance, in which the other elements are embedded, appears homogeneous or possibly finely granulated, with a varying opacity which is usually slightly greater than the surrounding cytoplasm (Fig. 19).

The membranes characteristic of the Golgi apparatus, the γ -cytomembranes, are arranged in pairs varying in number from two to six pairs (Fig. 19, 20). In some places in a Golgi zone, all the membranes may be seen in parallel arrangement and intimately related to one another, but more commonly a few membranes run in parallel rows and are separated from the rest by vacuoles. In this study, the term "membrane pair" refers to those membranes whose ends are fused together. Such fusions appear both at the periphery of a Golgi zone and more centrally (Fig. 20). The membranes of a pair are often separated by vacuolar spaces which are usually oval or fusiform (Fig. 19, 20). They vary in size from scarcely observable widenings of the intermembranous space to those having a cross diameter of about $0.6\ \mu$ and a length of about $1\ \mu$. In these vacuolar spaces, round or irregularly shaped bodies very often occur which appear homogeneous and, as a rule, have a pronounced opacity (Fig. 19, 20). The diameter of these bodies varies between 300 and 800 Å. Where there are no vacuoles the distance between the two membranes in a pair varies from 50 to 200 Å. A very constant interspace is found between the two adjacent membranes from neighboring pairs. This constant value is used in the determination of the dimensions of the membranes. Measurements were made of the total width of a false pair and of the distance between the centers of the membranes. From the values thus obtained, 180 ± 4 Å (SD=14, Tab. 3) and 120 ± 2 Å (SD=8, Tab. 3) respectively, the width of the separate membranes is calculated to be about 60 Å and the space between them also about 60 Å. No connections or anastomoses between the different pairs are observed.

The small vacuoles or vesicles occurring throughout the Golgi zone are characterized by an outer layer (about 60 Å in width) of the same opacity as the membranes and a less opaque inner structure (Fig. 19, 20). Near the membranes the vacuoles often appear in a row (Fig. 19, 20) and are elongated with the long axis parallel with the membranes, while in other parts they are more rounded. The width of the elongated vacuoles is about 200 Å and that of the rounded vacuoles varies from about 200 up to about 400 Å.

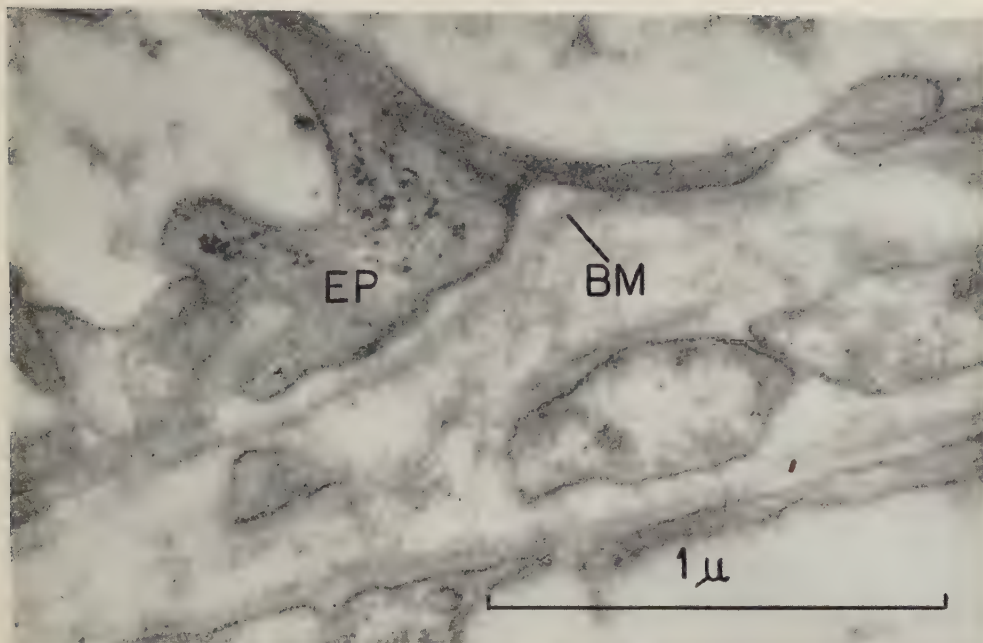


Fig. 16. Section through foot-like projections of adjacent epithelial cells showing narrow spaces between the projections. BM=basement membrane. Magnification 61,000 $\times$.

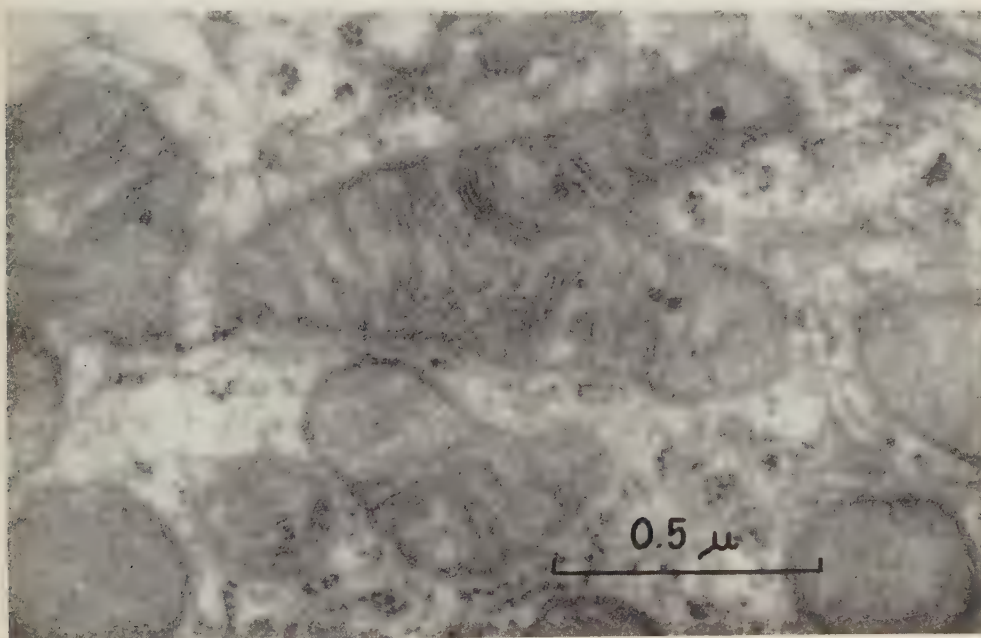


Fig. 17 Section through several mitochondria showing different arrangement of the inner membranes. Magnification 72,000 $\times$.



Fig. 18. Section through mitochondria showing outer and inner double-contoured membranes and opaque areas between the inner membranes. Between the mitochondria α-cyto-membranes (α-cy) pair-wise arranged in rows and granules (CG). Magnification 77,000 $\times$.

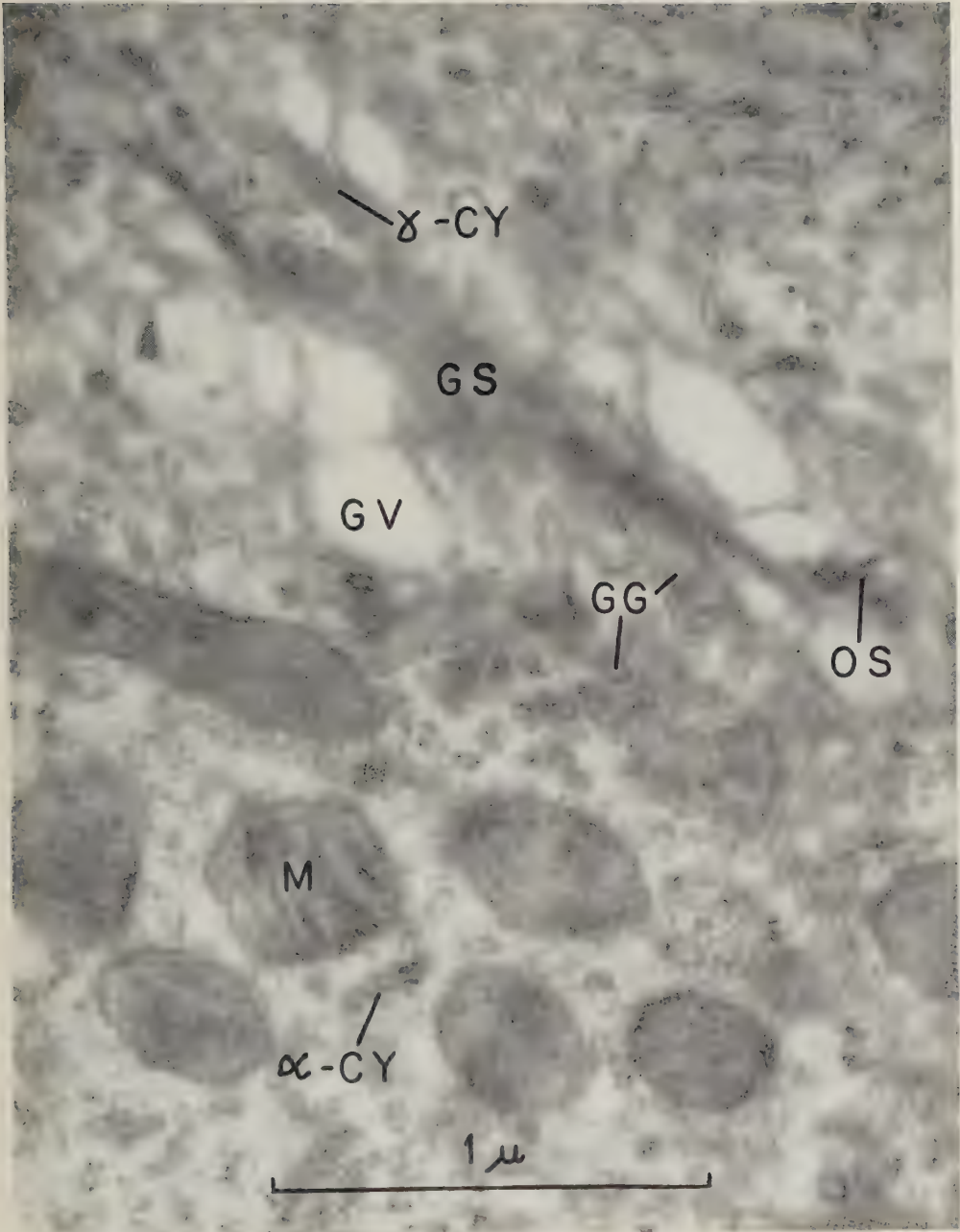


Fig. 19. Picture showing Golgi apparatus with ground substance (GS), γ -cytomembranes (γ -cy), vacuolar formations (GV), often containing highly opaque substance (OS), and small vesicles or vacuoles (GG). M=mitochondrion. α -cy= α -cytomembranes. Close to the upper part of mitochondrion (M) is observed an aggregate of small opaque granules. Magnification 60,000 $\times$.

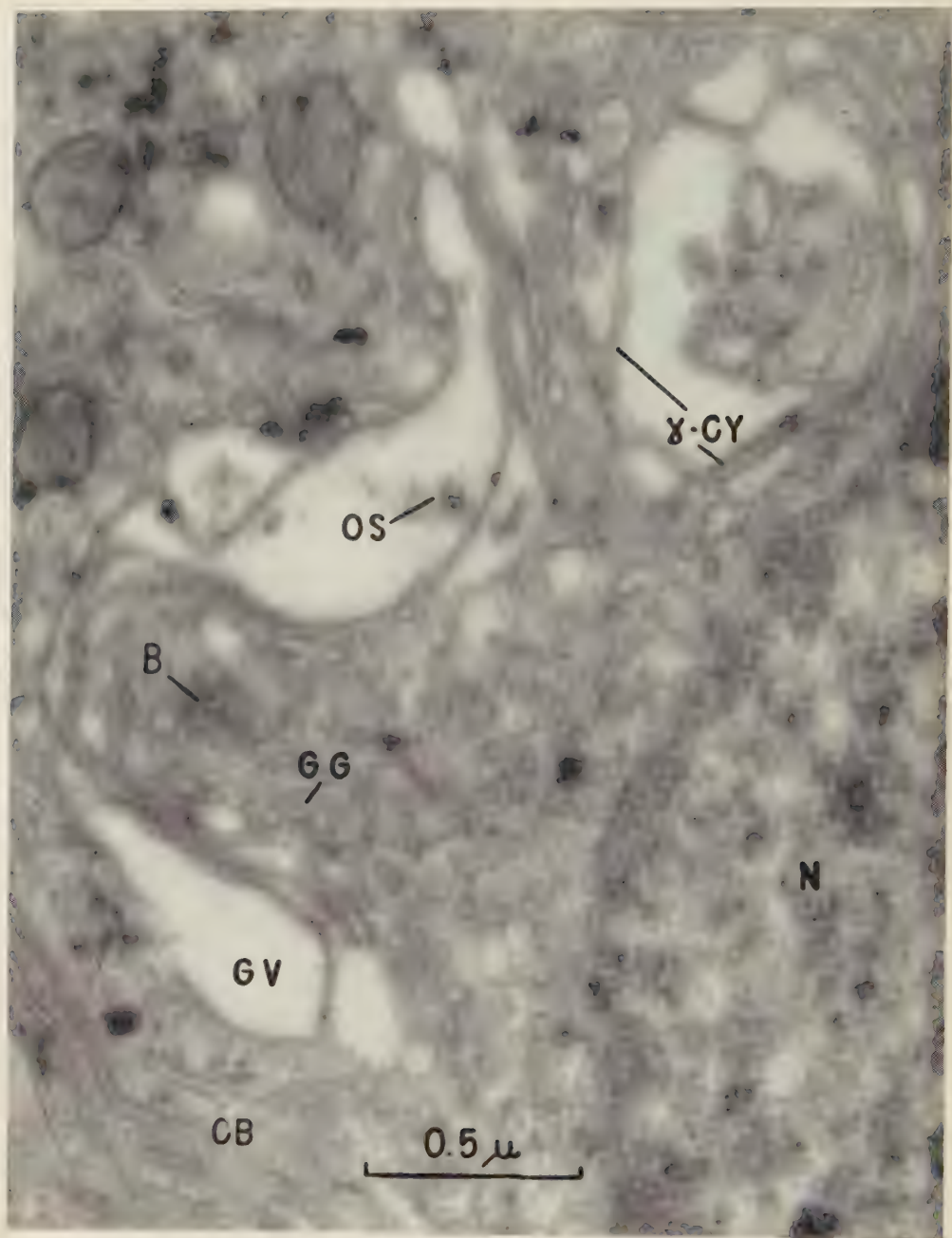


Fig. 20. Picture of nucleus (N) and Golgi apparatus with membranes (γ -cy), vacuolar formations (GV), containing opaque substance (OS), and vesicles or vacuoles (GG). B=vacuole-containing body in which the vacuoles are closely packed. CB= cell boundary. Magnification 61,000 $\times$.

Discussion.

By its specific structure the Golgi apparatus is an easily identifiable component in the absorbing cells of the jejunal epithelium. Its two main structural elements, the membranes arranged in pairs and the characteristic small vacuoles, are embedded in a ground substance in which other cytoplasmic components do not occur. The membranes of a pair are usually separated by vacuolar spaces of varying shape and size, which contain a highly opaque granular substance.

Thus, in the cells in this region, the Golgi apparatus shows an organization that is largely consistent with the one described for the secretory cells of the pancreas, the proximal convoluted tubule cells of the kidney, the epithelial cells of the epididymis and other cell types.

The correspondence between the different cell types with respect to the number and the dimensions of the membranes and the distances between adjacent membranes is striking. There is, however, some descriptive difference with regard to the arrangement of the membranes. Those membranes which intimately related to each other run through a Golgi zone by earlier authors are regarded as a pair (SJÖSTRAND and HANZON 1954 b, c) or as a unit (DALTON and FELIX 1954, RHODIN 1954). These classifications do not seem to be quite adequate, since they imply that one membrane on each side of the others will be without a partner. Hence, a pair describes more aptly those membranes which fuse together, even though, because of vacuolar spaces, the distance between them is only very rarely as constant as the distance between the adjacent membranes from different pairs.

Regarding the γ -cytomembranes, PALAY and PALADE (1955) have suggested that they are organized in the same way as the α -cytomembranes. This opinion, however, cannot be confirmed by this investigation because no branching or anastomosing between the different γ -cytomembranes can be observed, which is the case regarding the α -cytomembranes.

The small vacuoles (diameter up to 400 Å) found in the intestinal Golgi apparatus seem to correspond to the structures which in the pancreas (SJÖSTRAND and HANZON 1954 b, c), and epididymis (DALTON and FELIX 1954) are described as granules, in the kidney proximal tubule cells (RHODIN 1954) as vacuolar formations, in the liver cells (FAWCETT 1955) as small vesicles, in neuron cells (PALAY and PALADE 1955) as circular profiles and in different other cell types (HAGUENAU and BERNHARD 1955) as granules or microvesicles. All these different structures have about the same dimensions varying between 200 and 600 Å, with the exception of the pancreatic granules, the dimensions of which vary from 40 Å to 0.5 μ , the size of zymogen granules.

Regarding an eventual correlation between the γ -cytomembranes and the small vacuoles, the arrangement of elongated vacuoles often seen in prolongation of the membranes suggest the possibility that they develop from the membranes through some kind of budding as proposed by several authors (SJÖSTRAND and HANZON 1954 b, c, PALAY and PALADE 1955, BURGÓS and FAWCETT 1955).

The Golgi apparatus in the intestinal epithelial cell as well as in the liver cell (FAWCETT 1955) shows a structural difference in comparison to other examined cell types, in that the vacuolar spaces, which separate the membranes in a pair, contain a dense granulated substance.

Because of the constancy in the structure of the Golgi apparatus in different cell types, and because it remains unchanged in spite of variations in the pH and the osmolar concentration of the fixative (see the respective chapters), it is quite probable that the Golgi apparatus is a preformed cellular component and not an artefact as, for instance, myelin figures. This view is further supported by the fact that it is invariably situated adjacent to the distal pole of the nucleus.

Through light microscopic studies, a close structural relationship between the Golgi apparatus and other cell components, such as the nucleus and the mitochondria, has been pointed out. In the present study no obvious topographic relationship have been observed. A cytoplasmic layer of varying width can always be demonstrated between the Golgi apparatus and the nucleus, and as a rule, also between the Golgi apparatus and the mitochondria.

THE CYTOPLASM.

Earlier observations.

With the ordinary light microscope investigation of living cells no structures other than mitochondria and organelles of similar or greater dimensions can be distinguished in the cytoplasm which appears transparent and homogeneous. If the contrast conditions are changed, however, by using dark field or phase contrast microscopy, large and small particles may be seen.

Further details of the cytoplasm can be observed in fixed and stained material, but it is difficult or impossible to ascertain the true nature of the cytoplasm by light microscopic techniques.

Investigations by means of the electron microscope have been carried out on whole cells in tissue cultures and on sections from different cell types.

Examination of whole cells in tissue cultures — a method which unfortunately permits only very low resolution — was introduced by PORTER,

CLAUDE and FULLAM (1945). In the cytoplasm from chick embryo cells they observed a granular component, 300 to 1,500 Å in diameter, and a "lace-like reticulum" which appeared to be distributed over the larger part of the cell. They further saw vesicle-like bodies with a diameter of 1,000 to 1,500 Å in association with this network. Identical components were also found in sarcoma and carcinoma cells (PORTER and THOMPSON 1947, 1948). However, the electron microscopy of unsectioned material failed to contribute to a more exact definition of the morphology of the cytoplasmic structures.

The first observations of cytoplasmic components in sectioned material, which were carried out at a low resolution, showed the occurrence of a "fibrous or membranous component" (HILLIER 1950, HILLIER and GETTNER 1950) in the cytoplasm of liver cells.

These fibrils were also observed in the liver cells by DALTON, et al., (1950) and were said to have a diameter of 700 Å. These authors also assumed that this structure was identical with the basophilic substance seen in routine light microscopy preparations. The analogous distribution in the cells and the simultaneous reduction of fibrils and basophilic substance during starvation were interpreted as evidence in favour of this assumption. They also pointed out the possibility that the fibrils were identical with the structures in sarcoma cells observed by PORTER and THOMPSON (1947).

Similar structures were demonstrated by DALTON (1951 b) when investigating the chief cells of the stomach and the secretory cells of the pancreas, but were at that time described as a system of lamellae.

BERNHARD, et al., (1951, 1952) described the same structure in cells from liver, pancreas and salivary glands. They interpreted it as fibrillar and stated that it corresponded to the ergastoplasm observed in the light microscope. Thus, in accordance with DALTON, these authors assumed that the structure represented the basophilic components of the cytoplasm.

The organization of the structure under discussion has also been described as "canalicular" (PALADE and PORTER 1952) and as made up of "sacs" (J. WEISS 1953) in which the thickness of the wall was estimated at about 250 Å.

Further analysis of whole cells from tissue cultures led PORTER and KALLMAN (1952) and PORTER (1953) to the opinion that the network in the ground cytoplasm — then called "endoplasmic reticulum" — consisted of a system of minute canals and vesicles, in which the defining membrane was said to be of the same thickness as the cell membrane, that is, about 80 Å. The existence of a reticulum in living cells was verified by phase contrast microscopy.

Using an improved method of sectioning which permitted a resolution of better than 30 Å, and a modified technique of fixation, SJÖSTRAND (1953 a, b) and SJÖSTRAND and HANZON (1954 a) analyzed the cytoplasmic organization in the mouse pancreas both from freeze-dried and from osmium-fixed material. The cytoplasm was described as being made up of a highly developed system of concentrically arranged membrane pairs — so-called “intracellular cytoplasmic membranes”. This structural organization was also confirmed by investigation with intravital polarization optical microscopy (SJÖSTRAND 1953 b). By the fusion along their edges a membrane pair enclosed narrow spaces. The membrane was found to be about 40 Å in thickness, and the side of it facing the intermembranous space was smooth, while the outer side was covered with tightly packed angularly shaped granules with an average diameter of 140 Å. SJÖSTRAND (1956 b) has suggested the name α -cytomembranes for this type of membranes associated with granules. Granules of the same appearance and size as those attached to the membranes were also seen occurring freely in the cytoplasm.

Free granules with a diameter of about 40 Å were observed in the inner segment of the retinal rods (SJÖSTRAND 1953 c) and in the proximal tubule cells of the mouse kidney (SJÖSTRAND and RHODIN 1953, RHODIN 1954). The general occurrence of granules in the cytoplasm, 80 to 300 Å in diameter, was demonstrated by PALADE (1953 b, 1955), who found that they were particularly abundant in cells with basophilic reaction. He further supposed that they were identical with the fraction obtained by differential ultracentrifugation, which has been found to be rich in nucleic acids.

PORTER (1954) and PALADE and PORTER (1954) after examination of sectioned cells of different types (including cells from tissue cultures) concluded that the structure described earlier by PORTER as “endoplasmic reticulum”, was a cytoplasmic component occurring in most cell types. Judging from the pictures of tissue sections, the “endoplasmic reticulum” was said to consist of communicating tubules and vesicles which were separated from the rest of the cytoplasm by a membrane about 60 Å in width, which was sometimes covered with granules 80 to 300 Å in diameter on the outer surface. The structures occurring in the liver cells and other secretory cells were described as components of the “endoplasmic reticulum” arranged to form thin flat vesicles called “cisternae”.

Another type of cytoplasmic membranes was described by SJÖSTRAND (1953 b), SJÖSTRAND and RHODIN (1953), and RHODIN (1954) in the proximal tubule cells of the mouse kidney. It has also been seen in the cells of the ciliary body of the eye (HOLMBERG 1955). It was found that these membranes consisted of folds formed by the basal part of the cell membrane.

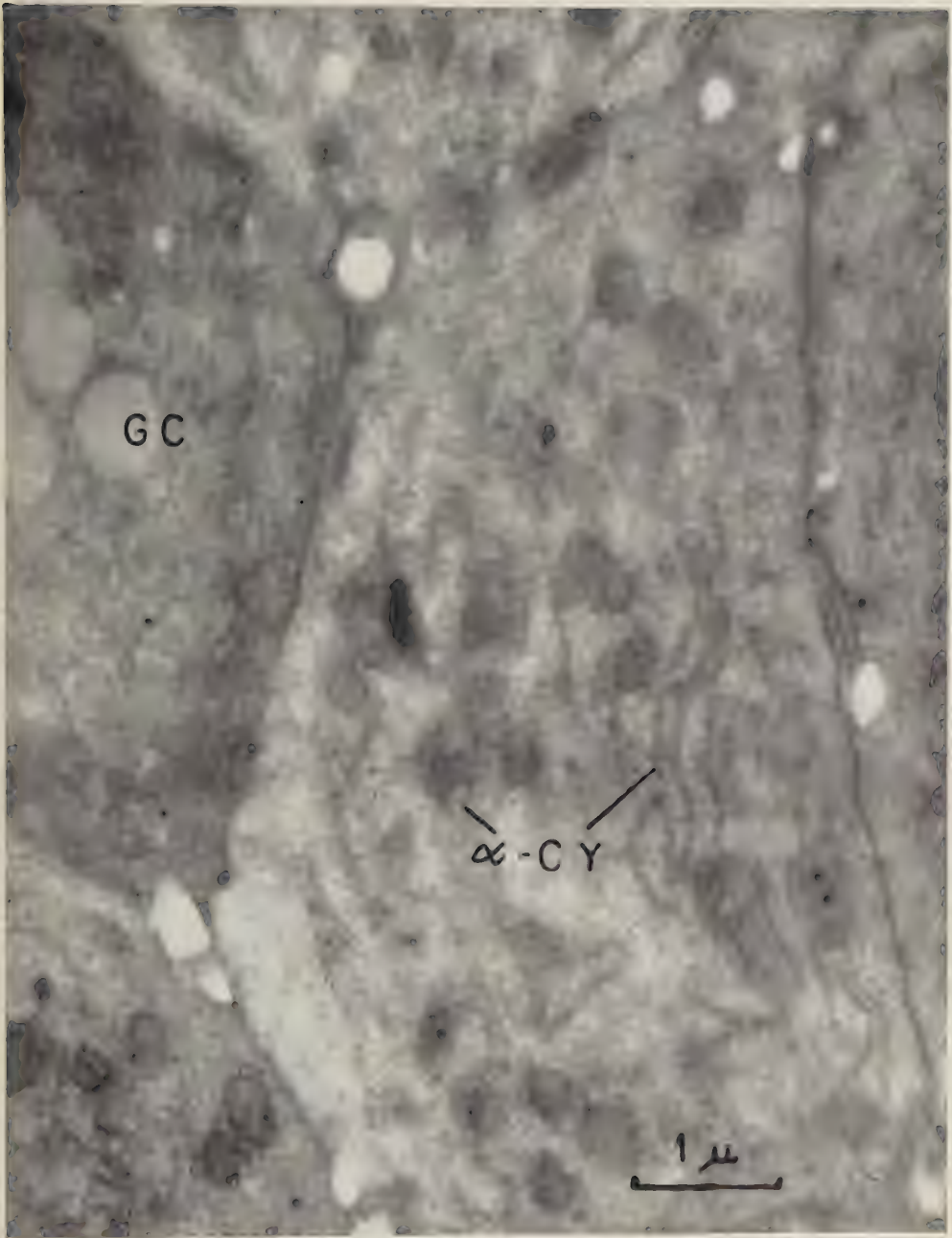


Fig. 21. Survey of cross sectioned cells showing the α -cytomembranes (α -cy) arranged in a thin network. GC: goblet cell
Magnification 20,000 $\times$

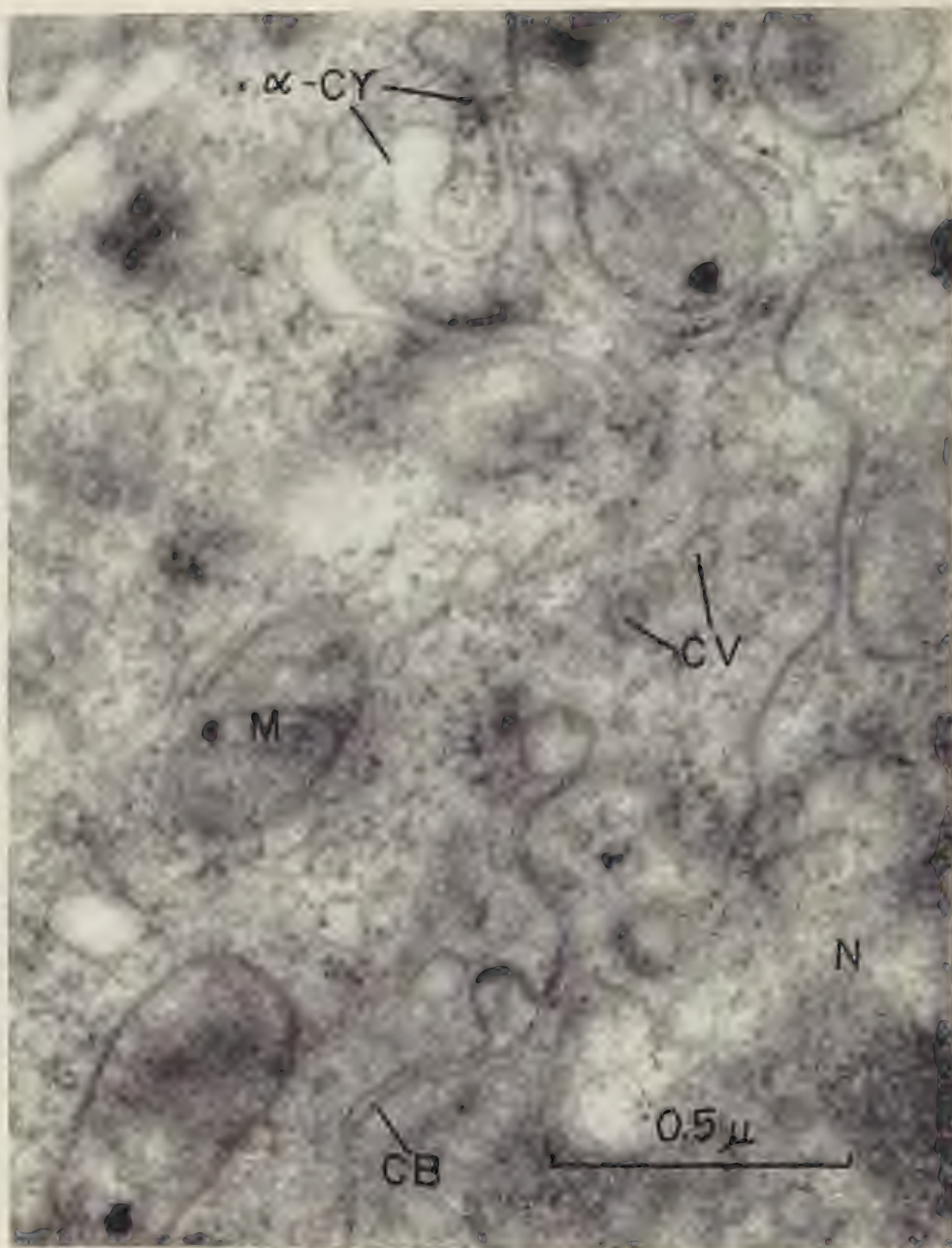


Fig. 22. Picture showing α -cytomembranes (α -cy), in some places dilatated to vesicle-like formations, cytoplasmic vesicles (CV), mitochondrion (M), cell boundary (CB) and nucleus (N) containing granules of about the same dimensions as those of the cytoplasm. Magnification 80,000 $\times$.

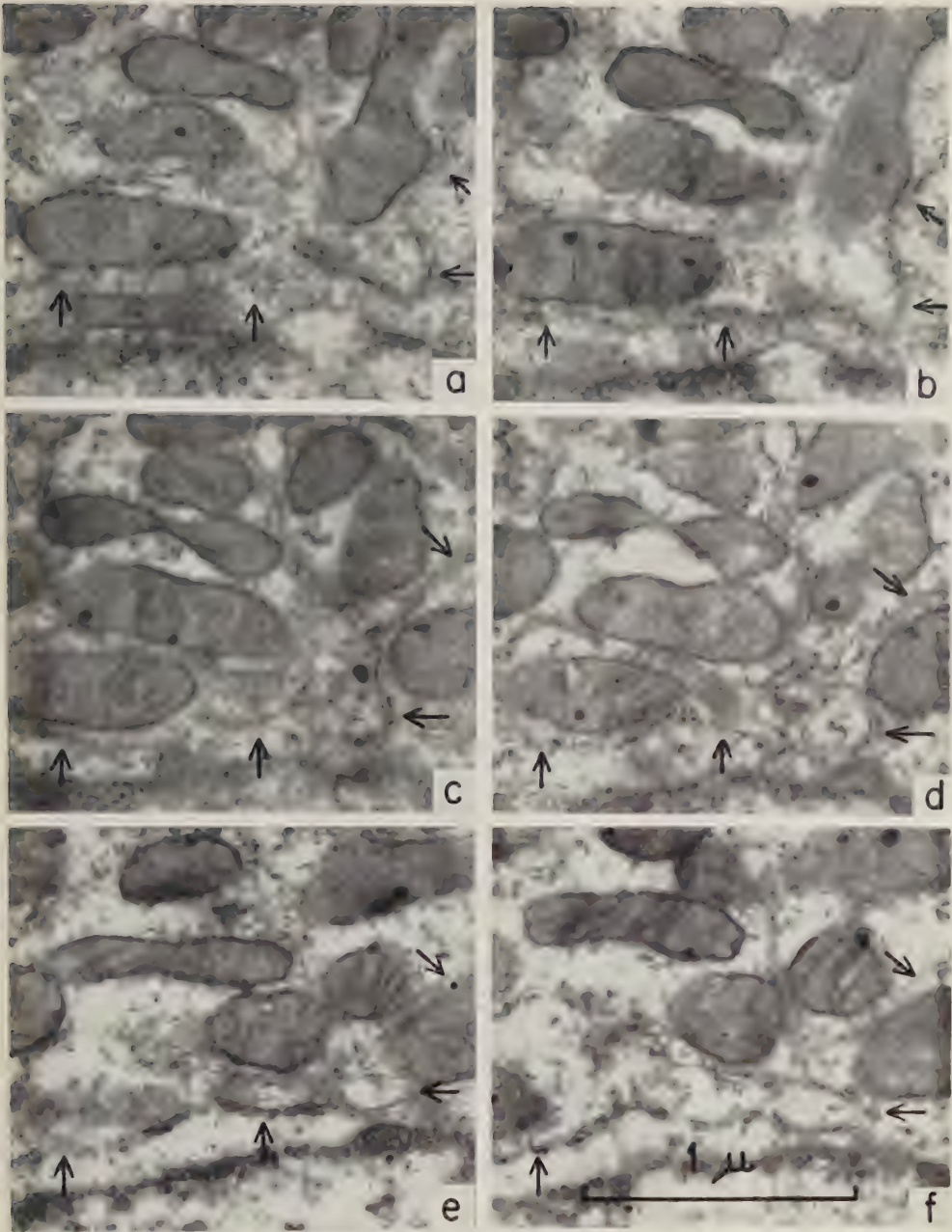


Fig. 23. Serial sections of α -cytomembranes (arrows) and mitochondria showing the parallel arrangement of the α -cytomembranes, which are not considered canalicular or tubular structures.
Magnification 37,000 $\times$.

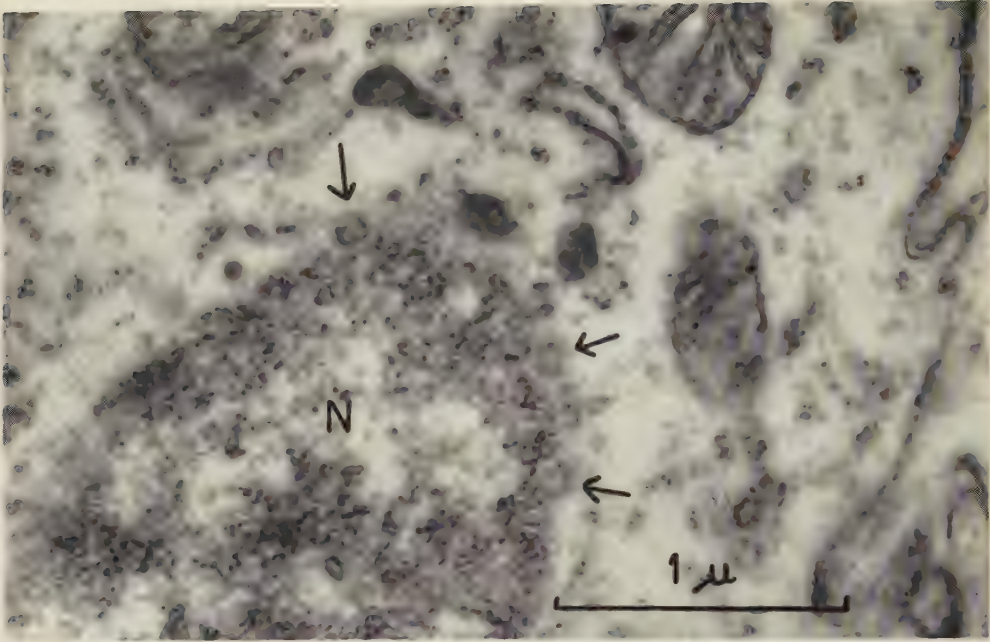


Fig. 24. Section of epithelial cell nucleus (N) showing round structures in the tangentially cut nuclear membrane (arrows) with an outer denser layer and a less dense center. Magnification 39,000 $\times$.

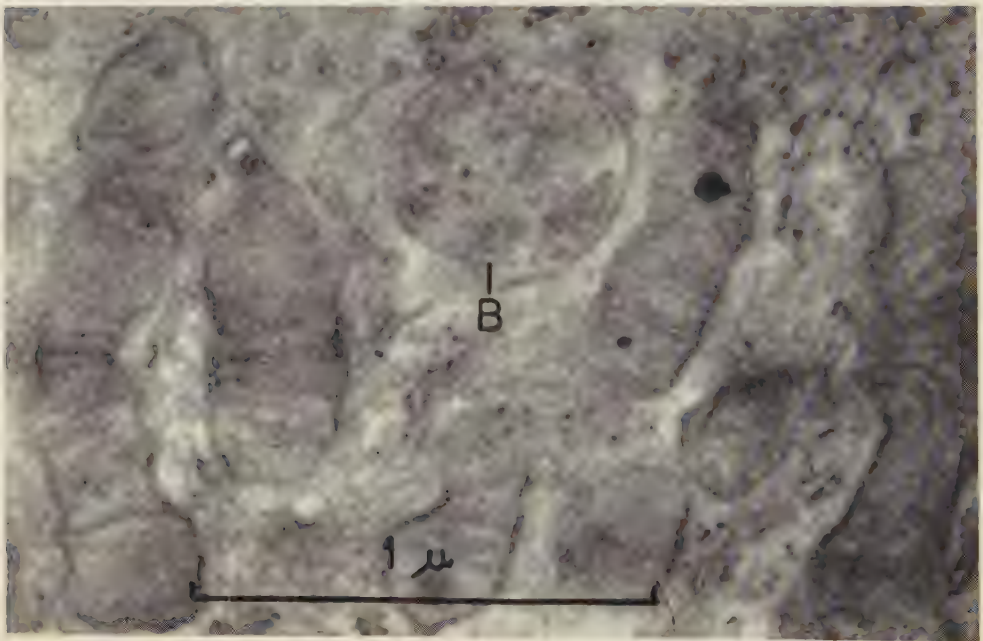


Fig. 25. Picture of mitochondria and vacuole-containing body (B) with a single surrounding membrane which at B seems discontinuous. Magnification 62,000 $\times$.

Membranes of this type have been named β -cytomembranes by SJÖSTRAND (1956 b).

A third membrane type has been found in the Golgi apparatus (SJÖSTRAND and HANZON 1954 b, c, DALTON and FELIX 1954) and in the organ of Corti (ENGSTRÖM and SJÖSTRAND 1954). These are called γ -cytomembranes (SJÖSTRAND 1956 b) and are described in the chapter on the Golgi apparatus.

Vacuolar structures occurring in the cytoplasm have been observed in the inner segment of the retinal rods (SJÖSTRAND 1953 c), in the cytoplasm of the cells of Schwann (DE ROBERTIS and BENNETT 1954) and also in other cell types (PALADE 1953 c, PALADE and PORTER 1954). PALADE and PORTER described these vesicles to be still another component of the "endoplasmic reticulum".

Results.

In the cytoplasm of the intestinal epithelial cells four different components can be distinguished in addition to the mitochondria, the Golgi complex and other organelles, which are described in separate sections. These four components are: a homogeneous ground substance, α -cytomembranes, free granules and vesicles. These components are rather evenly distributed except for the zone in which the brush border "rootlets" are seen, where the ground substance occurs but not the other cytoplasmic components (Fig. 8).

The ground substance of the cytoplasm has a rather low opacity after fixation in solutions isotonic to blood and appears structureless in pictures with a resolution of better than 30 Å. As is shown in experimental studies, the opacity varies with the tonicity of the fixative, thus diminishing in hypotonic and increasing in hypertonic solutions. These variations, however, are rather moderate and the opacity of the ground substance is always less than that of the mitochondrial ground substance regardless of fixative tonicity.

The α -cytomembranes (Fig. 18, 23) are built up of two components as SJÖSTRAND and HANZON have described: a slightly opaque membrane about 50 Å in width and granules, about 160 Å in diameter, attached to one side of this membrane. The membranes are arranged in pairs with the smooth, agranular sides facing each other. In the cells under discussion the distance between the membranes belonging to a pair is 280 ± 9 Å ($SD=40$, Tab. 3). In places, however, the intermembranous space is widened into vesicle-like formations (Fig. 22), with a diameter of up to 0.1 μ . The intermembranous space has about the same opacity as the surrounding cytoplasm from which it seems to be separated due to the fusion of the edges of the two membranes belonging to the pair. The spaces delimited by the

α -cytomembranes in the region between the brush border and the nucleus are mainly oriented parallel with the long axis of the cell. In the basal parts of the cell they are more irregularly arranged. The different intermembranous spaces seem to communicate with each other in many places. The distance between the membrane pairs varies up to 3 μ . The thickness of the single membrane exclusive of the granules is 50 ± 2 Å (SD=9, Tab. 3).

The granules attached to the outer surface of the membranes are mostly irregularly rounded, but triangular and rectangular forms also occur. The mean diameter of the granules is 160 ± 6 Å (SD=28, Tab. 3). The distance between the granules on the membrane varies from 150 to 450 Å. They seem to occur less frequently or may even be absent on those parts of the membranes which form the walls in the vesicle-like formations. No inner structure has been seen in the granules.

Free granules of the same size (mean value 150 ± 5 Å, SD=23, Tab. 3) and configuration as those attached to the membranes also occur in great numbers in the cytoplasm, where they often are seen aggregated in groups of 2 to 5 granules (Fig. 18).

The fourth structure observed in the cytoplasm consists of rounded vesicles delimited by a membrane about 50 Å thick, with a ground substance of about the same opacity as that of the cytoplasm (Fig. 22, 38). The diameter of the vesicles varies from 300 up to 1,000 Å with a mean value of 780 ± 50 Å (SD=170, Tab. 3). That these structures are vesicular formations and not merely distensions of the space between the α -cytomembranes has been demonstrated by serial sectioning. The vesicles seem to be especially abundant in a zone close to the brush border "rootlets".

Discussion.

In the cells from the intestinal epithelium the two cytoplasmic components which form the α -cytomembrane, i.e., membranes and the attached granules, have the same structure and dimensions as those occurring in the secretory cells of pancreas as described by SJÖSTRAND and HANZON (1954 a). The arrangement of two α -cytomembranes running closely parallel with the smooth, agranular sides adjacent to each other is the same in both cell types. The distance between two such membranes in a pair is 70—700 Å in the pancreas cells and 150—400 Å in the intestinal cells. The two cell types also differ in the distance between pairs, the interspace between the pairs in pancreas cells being estimated at 150 to 1,000 Å compared with a value of up to 3 μ in the absorbing cells of the intestinal epithelium.

Granules of the same type and dimensions as those attached to the mem-

branes also occur freely in the cytoplasm in great number and are often arranged in groups. A comparison between the secretory cells of the pancreas and the intestinal absorbing cells shows that these free granules in the former cells constitute a small percentage of the total number, whereas in the intestinal cells most granules occur free without connection with the membranes.

Granules of these macromolecular dimensions are believed (PALADE 1953 b, 1955) to constitute a component of the cytoplasm in most cell types. There are also smaller granules with a diameter of about 40 Å, which have been demonstrated by SJÖSTRAND (1953 c) in the inner segment of the retinal rods and by SJÖSTRAND and RHODIN (1953) in the proximal tubule cells of the kidney.

Vesicles are the fourth component of the cytoplasm described in this chapter. They appear as thin membranes delimiting a ground substance of about the same opacity as the surrounding ground substance of the cytoplasm. A similar description is given for the vesicles (also called granules or elongated bodies) which have been seen in connection with the membranes of the Golgi complex. Vesicles of this type are also described by PALADE and PORTER (1954, 1955), who suggest that the vesicles are pinched off from the membranous components of the "endoplasmic reticulum". In this investigation, however, no correlations between the spaces delimited by α -cytomembranes and the cytoplasmic vacuoles have been observed.

Regarding the question of the origin of these vesicles or vacuoles it can be mentioned here that investigations of post-mortem changes have shown that these vesicles are larger in specimens fixed 5 to 10 minutes after death than in those fixed within 2 minutes. Thus, it is possible that they are artefacts which are formed post mortem. Another possibility is that they represent granules which have been dissolved during fixation and embedding.

The structures which occur in the cytoplasm of cells have been called components of the "endoplasmic reticulum". This term was introduced by PORTER and KALLMAN in 1952 and later (PORTER 1954) was defined as "a system of strands and vesicles associated in the form of a complex reticulum ... closed in a membrane". This ambiguous description has resulted in the misuse of the term "endoplasmic reticulum", since different authors have applied it to various structures or as a collective name for several components occurring in the cytoplasm. Since ultrastructural analysis has shown that the cytoplasm in most tissues is built up of relatively few well-defined simple structures, it seems important to describe the cytoplasm in terms of its components rather than by a collective term.

THE NUCLEUS.

Earlier observations.

In light microscopic studies the cell nucleus has been described as being surrounded by a thin membrane (COWDRY 1932) and containing a "delicate network of threads with a few coarse irregular chromatin clumps" (OPPEL 1904, quoted from COWDRY 1932). The nucleus was also found to contain one or more nucleoli.

The nuclear membrane was demonstrated clearly for the first time by CALLAN and TOMLIN (1950) in their electron microscopic study of isolated nuclei of amphibian oocytes. The membrane in these cells was described as being made up of two layers: an outer one consisting of a sheet of material with annuli evenly spaced over its area, and an inner layer which appeared continuous. The internal diameter of the annuli was found to be about 300 Å, and the distance between the pore centers about 800 Å. These observations were later confirmed by BOVEY (1951), BAIRATI and LEHMANN (1952) and GALL (1954), all of whom used the same technique on different materials.

In sectioned material BAHR and BEERMANN (1954) described pore-like structures in the nuclear membrane in cells from the midgut and the salivary gland from *Chironomus*, where the pores were found to penetrate both layers in the membrane. In nerve cells from the frog, DE ROBERTIS (1954) found a double-contoured nuclear membrane in which he observed circles with a core of low density and a denser surrounding ring. These structures were interpreted as pores probably situated in one of the layers. The diameter of the pores was estimated to be about 800 Å. In sea urchin oocytes, AFZELIUS (1955) described the nuclear membrane as being composed of two dense layers (about 90 Å in width) enclosing a less dense layer (about 140 Å in width). In transverse sections, discontinuous areas in the nuclear membrane were observed. These regions were usually rounded and had a diameter of about 1,000 Å. Covering this area a thin diaphragm was observed. From the edges of this area cylindrical structures were found projecting into the nucleoplasm (600 Å) and cytoplasm (200 Å). The thickness of the cylinder wall was about 250 Å. In the lumen of the cylinder on the inner side of the membrane, a central globule was sometimes observed, which seemed to be kept in place by a loose gel. Approximately 40 to 80 pore-like structures were observed per μ^2 .

In mammalian cells, the nuclear membrane was described by HARTMANN (1952, 1953) as being built up of two dense components, (80 to 120 Å in width) separated by a less dense layer (100 to 150 Å in width). No pores could be seen. A nuclear membrane of similar structure was described by SJÖSTRAND and RHODIN (1953) in the proximal tubule cells from the mouse

kidney. After further analysis of these tubule cells, however, RHODIN (1954) found ring-like structures in the nuclear membrane, which had a diameter of about 900 Å. Whether these consisted of pores or simply reinforcements could not be decided. SJÖSTRAND and HANZON (1954 a) found a single nuclear membrane in the secretory cells of the mouse pancreas. In association with this membrane they observed an additional membrane, which they interpreted as belonging to the cytoplasm. This membrane was covered by numerous granules on the side away from the nucleus. No pores in the nuclear membrane were seen. WATSON (1954) who also analyzed the nuclear membrane in cells of the pancreas, described a double nuclear membrane in which he observed pores formed by the connection of the two components in the membrane. These holes, which numbered approximately 10,000 per nucleus, had a diameter of 250 to 300 Å. These studies were subsequently extended to include cells from embryonic ectoderm, mesoderm and endoderm from rat, and protozoa and algae. Pores were observed in all nuclear membranes examined. Pores similar to those described by WATSON were also observed by FAWCETT (1955) in liver cells from the rat and by PALAY and PALADE (1955) in neuron cells also from the rat. In spinal ganglion cells from rabbits, DAWSON, HOSSACK and WYBURN (1955) found that the nucleus was surrounded by a membrane 600 to 1,000 Å in thickness, built up of several stratified layers. Disc-like nodes were observed penetrating this membrane. These contained a dark cortex, an intermediate lighter layer, and a central dark spot. The diameter of the whole structure was estimated to be 850 Å and the number per nucleus to be about 10,000. These "nodes" were described as "solid cylindrical units with thick walls and a center filled with material diffusing through the membrane".

The nuclear substance in cells from proximal tubule of the mouse kidney was described as granules by SJÖSTRAND and RHODIN (1953) and by RHODIN (1954) as built up of an osmium impregnated and a non-osmium impregnated portion, the former being found mainly in association with the membrane but also in small areas scattered over the cytoplasm. This osmium impregnated portion was made up of round or oval granules about 250 Å in length and 150 Å in width, with a smaller central part and a denser outer zone about 50 Å in width. A similar condition was also observed in the nucleoplasm of rat nerve cells by HARTMANN (1953). Here, the denser component was described as composed of irregular fibrous masses. In the secretory cells of the mouse pancreas, the nuclear substance was described (SJÖSTRAND and HANZON 1954 a) as being composed of particles of uniform size, with a mean diameter of 170 Å. These particles were found to have an internal structure, which indicated that they consisted of aggregates

of still smaller units. A nuclear substance made up of dense particles was also described by DE ROBERTIS (1954) in nerve cells from frogs, and by FAWCETT (1955) in liver cells from rats. The size of the particles in the liver cells was estimated to be 150 to 200 Å. FAWCETT also observed a larger granular component in the nucleus with a diameter of 800 to 2,400 Å.

In light microscopic studies, the nucleolus has been described as being built up of vesicles (KÖLLIKER 1867, and others), filamentous structures (CAJAL 1893, ESTABLE 1930, and others), or granules and vacuoles (MONTGOMERY 1899). After analysis by means of phase contrast microscopy, ESTABLE and SOTELO (1951) described two components: a filamentous one which they called "nucleolonema", and a homogeneous one, "pars amorpha". This description agrees well with the electron microscopic observations of BORYSKO and BANG (1951) and BERNHARD, et al., (1951), in which the nucleolus was described as consisting of branching or anastomosing filaments embedded in an amorphous matrix. In nerve cells, HARTMANN (1953) found a solid or vacuolated nucleolus but was not able to demonstrate any fibrous structure. In the nucleus of the liver cell, however, FAWCETT found a nucleolus composed of "a coarse strand (125 to 200 m μ in thickness) that branches and anastomoses to form a tight meshwork or tangled skein". The coarse strand was found to be made up of closely packed dense granules, 150 to 200 Å in diameter. In the nucleolus of various cells, BERNHARD, et al., (1955) confirmed earlier observations of two components corresponding to the "nucleolonema" and "pars amorpha", of ESTABLE and SOTELO and described both as being composed of granules with a diameter of 100 to 150 Å.

Results.

The nucleus in the absorbing cells of the intestinal epithelium, is found in the lower half of the cell and is elliptical or irregularly rounded with shallow indentations. It is surrounded by two opaque layers which are separated from each other by a less dense layer, the width of which varies between 100 and 300 Å. In determining the membrane thickness, a mean value of 50 ± 8 Å (SD=22, Tab. 3) was obtained for each of the membranes. On studying sections cut tangential to these membranes, annular structures are observed which have a diameter of about 1,000 Å. These structures have an opaque outer zone of about 200 Å in width and a less dense inner structure (Fig. 24). The distance between the annular bodies varies usually between 0.25 and 1 μ , but they may be found still closer together in the indentations. In sections oriented at right angles to the membranes, a structure probably corresponding to these ring-like bodies has also been observed in the form of discontinuities, 500 to 1,000 Å in

length. Whether these discontinuities are true holes in the membranes surrounding the nucleus or holes bridged over by a thin membrane could not be ascertained because of the small number of observations. At the margin of the "hole" an area of increased opacity is seen, which extends a few 100 Å into the cytoplasm as well as into the nucleus. It may be assumed that this area corresponds to the outer zone of the ring-like structure observed in tangential sections.

The chromatin in the nucleus consists of irregularly rounded opaque granules with an average diameter of 170 ± 8 Å (SD=24, Tab. 3). These granules are, on the whole, evenly distributed over the nucleus although some aggregate on the membrane and in some areas in the inner part of the nucleus. Aggregates of a few granules are also seen in many cells. In the large aggregates found along the nuclear membrane less dense areas of varying shape and size are often seen (Fig. 2, 14).

The nucleolus consists of an elongated aggregate of nuclear granules which in some nuclei may be tightly packed throughout the nucleolus, while in others may occur more sparsely, being arranged in filiform intercommunicating units, 0.1 to 0.2 μ in width. Occasionally it may be observed that these strands are oriented primarily in the longitudinal plane of the nucleolus. One end of the nucleolus is often seen in contact with the nuclear membrane. No membrane can be observed around the structure.

Discussion.

The structure of the nuclear membrane in the intestinal epithelial cells is, on the whole, quite similar to that described for the neuron cells (HARTMANN 1952, 1953), certain kidney cells (SJÖSTRAND and RHODIN 1953, RHODIN 1954), and liver cells (FAWCETT 1955), with two opaque components separated by a less dense space. As for the pores described by various authors in the nuclear membranes of different cell types, structures have been observed in tangential sections of intestinal epithelial cells which are similar in size and appearance to the ring-like structures observed by DE ROBERTIS (1954) in nerve cells from the frog and by RHODIN (1954) in kidney cells from the mouse. In transverse sections through the membranes it is difficult to analyze these structures in detail, mainly because of their small number but also because of the relatively dense arrangement of both cytoplasmic and nuclear components after fixation in isotonic solution. The observations that have been carried out, however, suggest a composition which is comparable with the one described by AFZELIUS (1955) for sea urchin oocytes, with a chimney-like structure in which the lumen is bridged over by a thin membrane. Regarding the functional significance of these structures, it can only be assumed that they may

represent areas which differ in their permeability properties from other parts of the membrane.

In these cells, the nuclear substance appears to be built up in the form of opaque granules which, in places, aggregate into larger units. This granular arrangement has been described earlier for several cell types. The nucleolus is also seen as a large granular aggregate occasionally arranged in filiform units corresponding to the so-called nucleolonema, and analogous to the structure described by FAWCETT (1955).

OTHER CELL COMPONENTS.

Besides the organelles described in the previous sections, two additional components are found in the intestinal epithelial cells. One of these components, which shows considerable variation in size, is characterized by a homogeneous ground substance containing small opaque granules and membrane structures. The other component, which is of constant size, contains vacuolar units.

Component I.

Results.

The first cell component of variable size has been observed in about 100 cases. As a rule, only one of these components appears in each although on a few occasions two have been encountered in the same cell. It is found in the area between the brush border and the nucleus, usually closer to the brush border. Occasionally it has been observed in the immediate neighborhood of the nucleus (Fig. 32). It is defined by a double-contoured membrane in which the opaque components and the intermediate less dense layer are about 60 Å in thickness, respectively. This body is more or less rounded in shape with a diameter that varies between 0.25 and 1 μ with a few as large as 4 μ . Its ground substance appears homogeneous or finely granular with an opacity that corresponds approximately to that of the ground substance of the mitochondria. It contains two different structures which occasionally seem to fuse together. One of these structures, observed in most of these bodies, consists of highly opaque granules with a diameter of 60 ± 2 Å (SD=2, Tab. 3), (Fig. 27, 28, 32). These granules may either be dispersed throughout the structure or regularly arranged in groups or rows (Fig. 28). When arranged in rows, the granules occasionally lie so closely packed that they seem to form confluent lines. The other type of structure, which occurs mainly in the larger units, consists of membrane structures which show a lower opacity than the granules. These membranes, which appear in some cases to be double-contoured, are of

about the same dimensions as the outer membranes of mitochondria and delimit round or elongated areas of the ground substance. Membranes oriented transversely to the long axis and likewise double-contoured can be observed in the inner parts of these areas which are 0.2 to 0.4 μ in width and up to 0.5 μ in length. In some cases the closely packed rows of granules may be continuous with the membranes bounding these areas (Fig. 28, 32).

Granules similar to those described above have also been found in the cytoplasm (Fig. 19) without association with any structure and on one occasion have been observed in a vacuole-containing body to be described below (Fig. 26).

Besides granules and membranes, the following structures have been observed in this cell component: vacuolar formations with diameters up to 0.1 μ (Fig. 32, 33) close to which small opaque areas appear and often seem to project into the vacuoles.

Larger, more irregularly shaped areas give the impression of the expulsion of parts of the entire structure into the cytoplasm (Fig. 29, 32). This pattern is observed in the largest of these cell components.

The same specimens used in this electron microscopic study have been sectioned at 0.6 μ and studied by phase contrast microscopy. In many cells, opaque round bodies were observed in the area between the brush border and the nucleus. They varied in size from barely discernible to approximately 4 μ . In the larger bodies lighter and darker areas of varying shape and size were observed (Fig. 45, 46).

Discussion.

The structure described above is often similar in size to mitochondria. Hence, in the light microscope it may possibly be mistaken for a mitochondrion, although in many cases it is much larger than this organelle and should be differentiated easily from a mitochondrion. On the other hand light microscopic studies have shown that the so-called cell center is located between the brush border and nucleus. This is the same area of the cell in which the structure described in this section is observed. These facts justify an attempt to correlate the light and electron microscopic observations.

The structure known as the cell center or cytocenter was first described by VAN BENEDEN (1883), after studying cell division in eggs of *Ascaris megalocephala*. This structure is situated in the center of the cell and built up of a small body, the centrosome, which is surrounded by a lighter zone, the attraction sphere or the astrophere. The centrosome, which may consist of one, two or several small bodies, has also been called the central

body, centriole, diplosome or microcentrum. VAN BENEDEN suggested that the centrosome gives rise to the "corpuscules polaires" situated at the poles of the spindle. This cell center has been found to occur in many kinds of cells (ZIMMERMANN 1894, 1898, M. HEIDENHAIN 1894, 1897, M. HEIDENHAIN and COHN 1897, and others). Demonstration of this structure, however, required certain special staining techniques the most important one being Benda-Heidenhain's iron-hematoxylin method. Its presence in the intestinal epithelial cells was demonstrated for the first time by ZIMMERMANN (1894, 1898) in preparations from the intestine of man. In this epithelium the cell center was usually found near the brush border, but occasionally also in the neighborhood of the nucleus. It was described as being built up of two small bodies lying close together, the diplosome, which were surrounded by a lighter zone, with a diameter that corresponds approximately to $\frac{1}{3}$ of the transverse diameter of the cell. ZIMMERMANN made no measurements of the two components of the diplosome but described each as "ein scharfes, minimales schwarzes Pünktchen", and it seems probable from this description that the order of magnitude would be $0.2\ \mu$, i.e., just at the resolving power of the light microscope. M. HEIDENHAIN (1894, 1897) described the centrosome in lymphocytes and bone-marrow giant cells as being composed of bodies smaller than $0.5\ \mu$ of varying shape, which were capable of growing and increasing in number by pinching off.

A comparison can be made between the cell center described in light microscopic studies and the cell component observed with the electron microscope and described here. The structure in both cases is found in the area between the brush border and the nucleus, usually nearer the brush border, but occasionally it has been observed in the immediate neighborhood of the nucleus. In light microscopic studies only one such structure is seen to appear in each cell. The structure demonstrated in this electron microscopic study of the intestinal epithelium has been observed in a sufficient number of cases to justify the assumption that it is an ubiquitous cell component which usually occurs singly. In this respect, too, the correlation appears to be satisfactory. On the other hand, comparison between the values which have been cited for the size of this structure shows certain differences. By light microscopy, the diplosome does not appear to exceed $0.5\ \mu$ in diameter, while the structure observed by electron microscopy is 0.25 to $1\ \mu$ in diameter, with some as large as $4\ \mu$. One explanation of this difference might be that with iron-hematoxylin only the diplosome is stained, while with osmium fixation the zone surrounding the diplosome is also stained. According to ZIMMERMANN, this zone is about $\frac{1}{3}$ of the transverse diameter of the cell, that is, an estimated size of $1\text{--}3\ \mu$, which seems to agree well with electron microscopic data.

This explanation is also rendered probable by light microscopic examination of specimens fixed with osmium and embedded in plastic. In several cases, structures have been observed which are quite comparable in size to the cell components demonstrated by electron microscopy (Fig. 45, 46). It may be proposed that the part of the structure situated centrally, in Fig. 28, which is built up of small granules arranged in rows, constitutes one diplosome component, while the surrounding less dense part, which contains membrane structures, corresponds to the zone which stains lightly with iron-hematoxylin. The central opaque component in Fig. 28 is about $0.1\ \mu$ in diameter and about $0.5\ \mu$ in length, values which satisfactorily agree with those stated for a diplosome component. The fact that the other half of the diplosome does not appear, is probably explained by the thinness of the sections.

Thus, from this comparison it appears probable that the structure observed with the electron microscope is identical with the so-called cell center. However, in order to establish definitely that this is the case, it seems necessary to study the appearance of these structures in cells under different phases of division, where the cell center in light microscopic studies has been found to play an important part. So far as intestinal epithelium is concerned, an analysis of cells in the intestinal crypts must be made.

With respect to the function and role of the cell center, several hypotheses have been proposed, among which the following may be mentioned: a dynamic center for cell division (BOVERI 1895), a coordination center for different forces acting in the cell (M. HEIDENHAIN 1897), an organ for the distribution of the chromatin in cell division (ZIMMERMANN 1898), a center of motor activity, e.g., in the filaments of the ciliated epithelium (ZIMMERMANN 1898), a center inducing protoplasmic contraction in secretory cells causing discharge of secretory products (ZIMMERMANN 1898).

This investigation permits no conclusions with regard to the function of this structure, although some pictures (Fig. 28, 29, 30, 31) seem to indicate that it is involved in the genesis and/or destruction of mitochondria.

Accumulations (diameter of about $0.1\ \mu$) of small opaque granules of the same size as those described above have been found on several occasions in the cytoplasm (Fig. 19). Once such an accumulation of granules was observed in the so-called vacuole-containing body (Fig. 26).

A similar structure has also been observed in epithelium from the urinary bladder (ZETTERQVIST and SHELDON 1956), cells from the ciliary body of the rabbit's eye (HOLMBERG 1956), and in secretory cells from the parotid glands in rats (RUTBERG 1956).

Results.

The other cell component described in this chapter is characterized by many small round vacuoles which are delimited by a single membrane and occur in a ground substance of varying density. Often several of these vacuole-containing bodies may be found in a single section and are located in the area between the nucleus and the brush border. They are usually round, but oval ones may also occur. Their size varies, with the smallest measured diameter being $0.3\ \mu$ and the largest $0.7\ \mu$. The smallest bodies, usually found close to the Golgi apparatus, contain vacuoles which are tightly packed and in some instances can be discerned only with great difficulty (Fig. 20). Closer to the brush border the bodies are larger and the ground substance less dense, the vacuoles being more scattered and hence more readily discernible (Fig. 25). The diameter of the vacuoles is $400 \pm 10\ \text{\AA}$ (SD=34, Tab. 3) and their surrounding membrane is about $50\ \text{\AA}$ in thickness. The membrane defining the vacuole-containing bodies seems to be continuous in the case of the smaller units, while around the larger ones it shows frequently interruptions (Fig. 25). This membrane has a width of $50 \pm 3\ \text{\AA}$ (SD=11, Tab. 3).

Discussion.

The cell component presented here as the vacuole-containing body is probably identical with the structure described by PALAY and PALADE (1955) as "a vesicular formation limited by a single membrane and filled with small circular profiles". However, the view advanced by these authors that the "circular profiles" would represent "membrane invaginations" cannot be accepted. A detailed description of this structure has been presented by RHODIN and DALHAMN (1956), who have found that it is a cell constituent of the tracheal epithelial cells from rats.

With respect to the function of this structure, nothing definite can be said at present, but it seems possible that it is identical with those structures which by combined histochemical and electron microscopic analysis have shown a positive reaction to acid phosphatase (SHELDON, et al., 1955). Both techniques have demonstrated a similar composition and location (in the area between the brush border and the nucleus) for these structures and thus favour this possibility. With the electron microscope, they are observed to be made up of round bodies containing vacuoles with an average diameter of $400\ \text{\AA}$, while in the preparations treated histochemically, grape-like clusters of round dense bodies, each body being about $500\ \text{\AA}$ in diameter, have been observed. Because of the comparatively poor fixation ob-

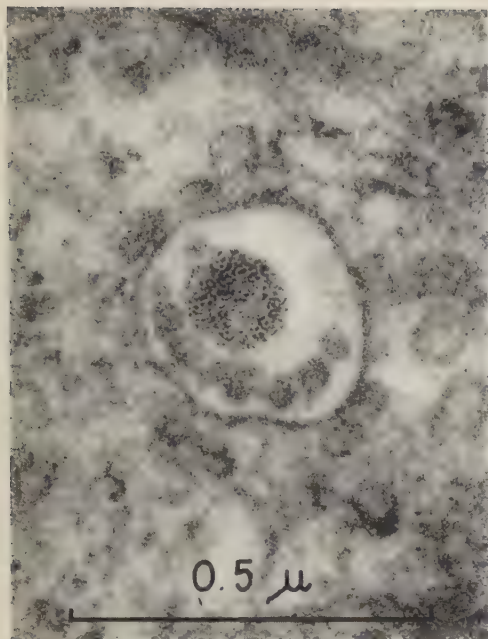


Fig. 26. Aggregate of small opaque granules and vacuolar structures within a vacuole-containing body. Magnification 95,000 $\times$.

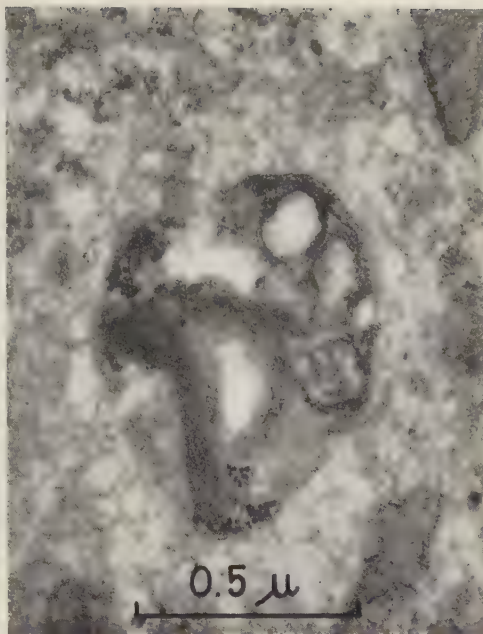


Fig. 27. "Component I" containing granules, membranes and "empty" areas. Magnification 59,000 $\times$.

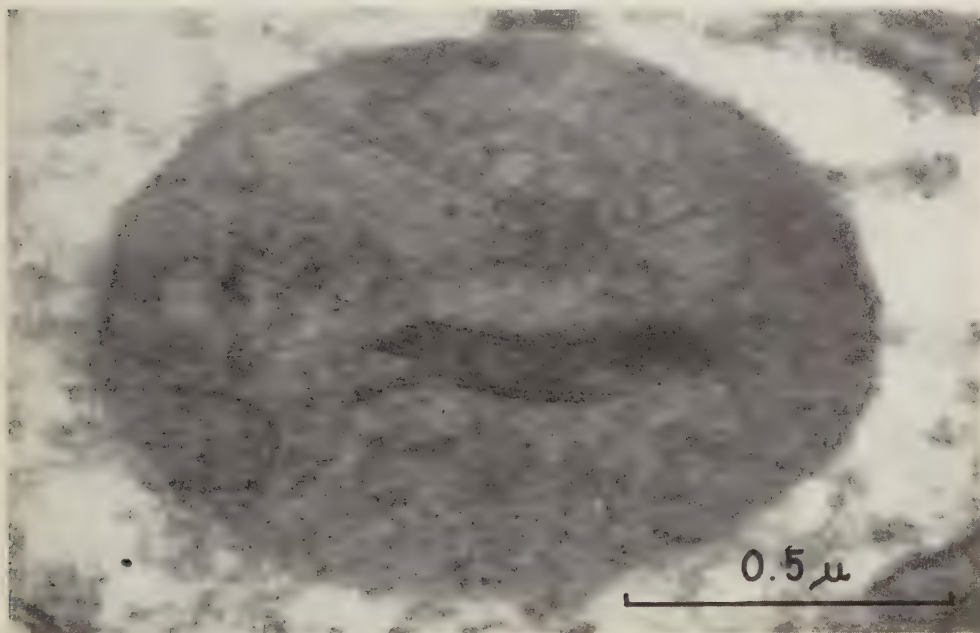


Fig. 28. Section of "component I" containing small opaque granules arranged in parallel rows. In other parts of the structure there are membranes delimiting elongated areas, one of which is connected with the granular rows. Magnification 86,000 $\times$.

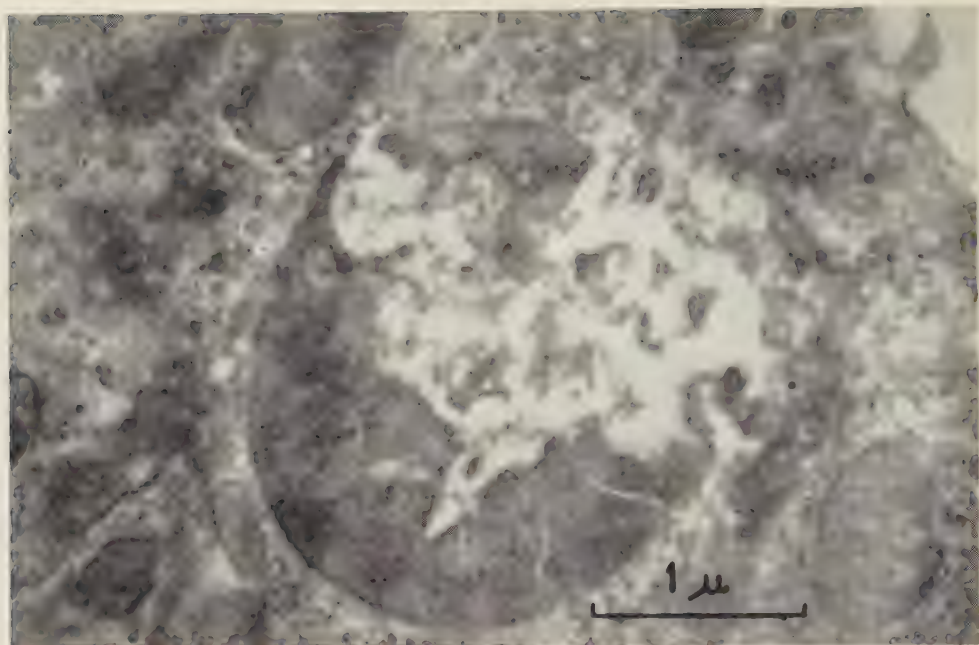


Fig. 29. Section of "component I" in state of disintegration. In the left part a round structure similar to a mitochondrion is observed. Magnification 28,000 $\times$.

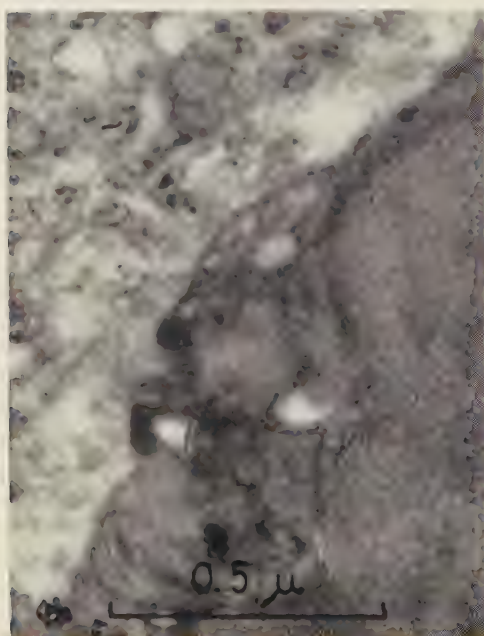


Fig. 30—31. Parts of "component I" containing double-contoured membranes of dimensions similar to the mitochondrial outer and inner membranes. Fig. 31 is a detail of Fig. 30. Magnification Fig. 30 72,000 $\times$, Fig. 31 51,000 $\times$.

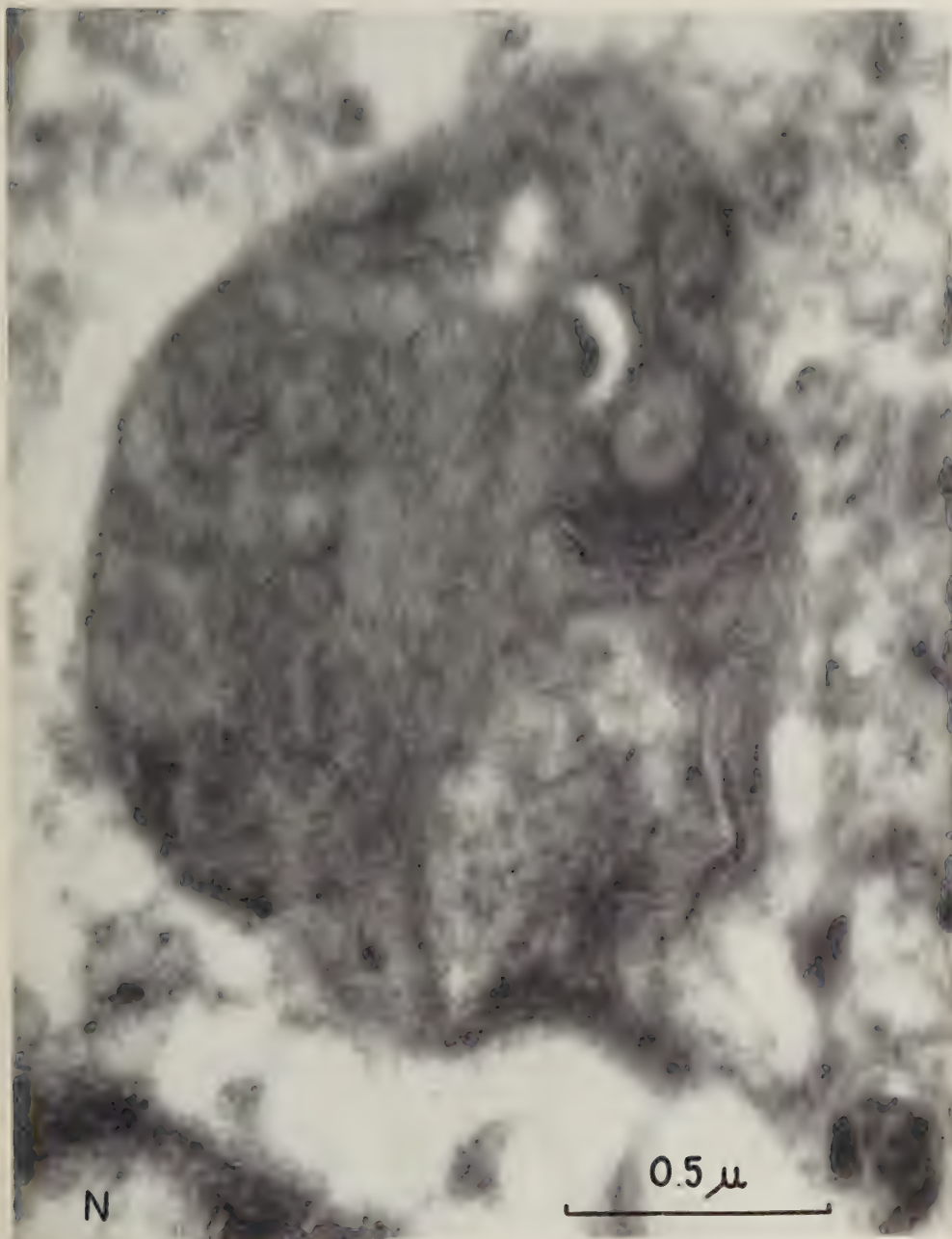


Fig. 32. Section of "component I" adjacent to the nucleus (N) containing small opaque granules arranged in parallel rows. In the upper right part some elongated areas are delimited by a vaguely defined membrane. In the lower right part of the picture there is open contact with the cytoplasm.
Magnification 72,000 $\times$.

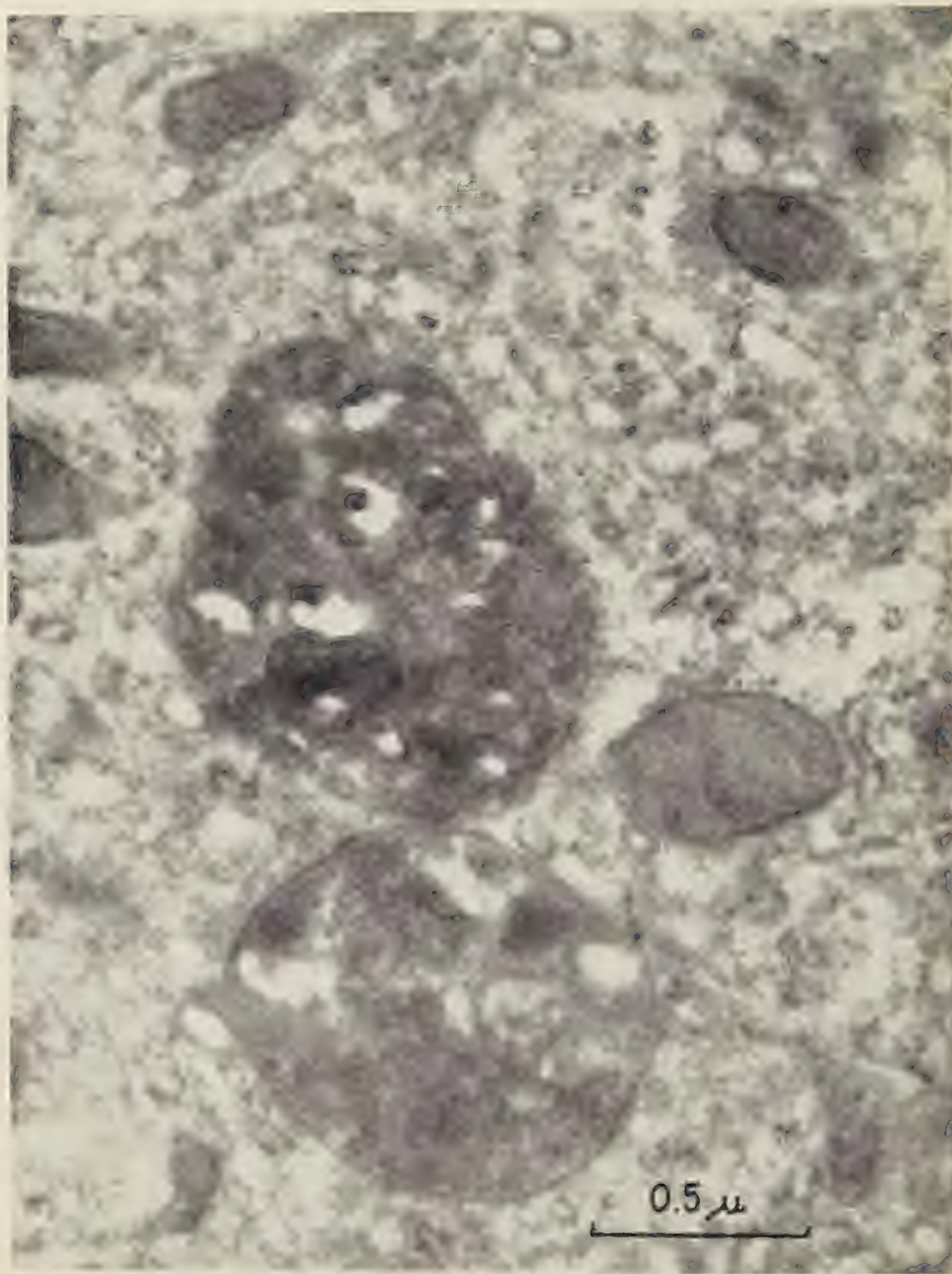


Fig. 33. Picture of section in which two "component I" are observed. In both "empty", irregularly shaped areas occur. Adjacent to or protruding into these areas opaque figures are seen. Magnification 62,000 $\times$.

tained in the histochemical preparations up to the present time together with the opaque metal precipitate on these bodies as due to the positive reaction, a more detailed analysis of these bodies is impossible.

It should be pointed out that these vacuoles in the vacuole-containing bodies are of about the same dimensions as the vesicles or vacuoles occurring freely in the cytoplasm.

III

FIXATION EXPERIMENTS

It is known from light and electron microscopical investigations of different tissues that fixation procedures often result in structural artefacts and cellular distortion. In addition, the sensitivity for such influences varies from one cell type to another.

In order to obtain a clearer understanding of these problems for the tissue under investigation, specimens have been studied after fixation at different times after death, in solutions of different osmolar concentrations and of varying pH.

POST-MORTEM CHANGES.

Earlier observations.

Light microscopic studies have shown that the first signs of cell injury are usually manifested by changes in the mitochondria (COWDRY 1932, DUTHIE 1935, ZOLLINGER 1948, and others), and that these appear within a short time after the supply of oxygen to the tissue has been interrupted. Descriptions have been given of how the mitochondria first swell, the rod-shaped type assuming a more rounded shape, then become vacuolized and finally disappear. In transplanted liver cells, DUTHIE found that the mitochondria had undergone swelling after 30 minutes, while ZOLLINGER, with the aid of phase contrast microscopy, saw swollen mitochondria in suspensions of liver cells after only a few minutes. ZOLLINGER was also able to show that the swelling of the mitochondria was dependent upon the temperature, since the reaction was slowed down at low temperature ($+2^{\circ}$ to $+4^{\circ}\text{C}$) and increased at 45°C , in comparison to the reaction at room temperature.

In systematic electron microscopic analyses of cell changes of this type, these observations have been verified for kidney tissue (RHODIN 1954) and for pancreatic tissue (SJÖSTRAND and HANZON 1954 d). Thus, in the proximal convoluted tubule cells of the mouse kidney, RHODIN found an indication of an increase in the width of the mitochondria within 5 minutes post mortem. After 15 minutes this increase was more pronounced and was accompanied by a vacuolization of the cytoplasm. The changes described above were marked at 60 minutes post mortem. On examination of pieces of tissue which had been fixed immediately after death, post-mortem changes were observed only in the deeper portions of the speci-

men, due to the slow rate at which the osmium tetroxide penetrates the tissue (about 40 μ in 5 minutes).

Results.

The animals used in these experiments were white mice which had been killed by decapitation. The bodies were kept at a temperature of $+1^{\circ}$ to $+2^{\circ}\text{C}$ for 5, 10, 20, and 40 minutes, respectively, before the abdomen was opened and pieces of tissue from the jejunum were taken in the usual way for fixation in a buffered blood-isotonic solution of 1 % osmium tetroxide.

The appearance of the cell structures 5 minutes post mortem (Fig. 34). The material consisted of 4 animals from which a total of 29 cells were analyzed. The mitochondria appeared, as a rule, more rounded than usual, and on measuring the width a mean value of $0.36 \pm 0.006 \mu$ ($\text{SD} = 0.031$, Tab. 4) was obtained. This differs from the value of $0.28 \pm 0.003 \mu$ ($\text{SD} = 0.029$, Tab. 4) for normal mitochondria. In virtually all mitochondria a destruction of the ground substance was observed along with the appearance of one or more small, irregularly shaped and ill-defined areas, the maximum diameter of which was usually smaller than half the width of the mitochondrion. The outer mitochondrial membranes appeared unchanged and were of normal dimensions (Tab. 5). The inner membranes were destroyed in the vacuolized areas but in other parts they were of normal appearance and dimensions (Tab. 5). In the cell cytoplasm, solitary rounded vacuoles were seen with a maximum diameter of about 0.2 μ . No changes were observed in the brush border, the cell membrane, the nucleus and the Golgi apparatus.

The appearance of the cell structures 10 minutes post mortem (Fig. 35). The material consisted of 2 animals from which 18 cells were analyzed. Here, the changes described above were accentuated with respect to the destruction of the mitochondrial ground substance and the inner mitochondrial membranes. In these cells the destroyed areas usually extended across the whole mitochondrion, and since several such areas often appeared in the same mitochondrion, a large part of the inner membrane system was destroyed. The outer membranes were well preserved. The width of the mitochondria was $0.37 \pm 0.003 \mu$ ($\text{SD} = 0.011$, Tab. 4). In the cytoplasm, vacuoles occurred which were larger and more abundant than those observed in the previous group, while the nucleus, the cell membrane, the Golgi apparatus and the brush border projections were of normal appearance.

The appearance of the cell structures 20 minutes post mortem. A total of 10 cells from 2 animals was analyzed. The changes were marked with considerable vacuolization of the cytoplasm and mitochondria and advanced destruction of the inner mitochondrial membranes. The outer membranes also seemed to be partially destroyed. The width of the mitochondria was $0.39 \pm 0.019 \mu$ (SD=0.060, Tab. 4). The nucleus showed disintegration of the chromatin, which accumulated toward the periphery. In the brush border projections, vacuoles were observed in places, but the cell membrane still appeared normal. In the Golgi apparatus, the vacuoles seemed to be larger than normal.

The appearance of the cell structures 40 minutes post mortem (Fig. 36). A total of 10 cells from 2 animals was studied. The increase in the width of the mitochondria from $0.28 \pm 0.006 \mu$ for the normal to $0.47 \pm 0.016 \mu$ (SD=0.059, Tab. 4) and the destruction of their inner membranes were marked. The outer membranes also were broken off in many places. The dimensions of those parts of the inner and outer membranes which were still preserved agreed with the normal values (Tab. 5). The vacuolization of the cell cytoplasm and the brush border projections was considerable, but the cell membrane on the free surface still remained unchanged and double-contoured. The nucleus showed disintegration of the chromatin, which accumulated toward the periphery. The vacuoles of the Golgi apparatus seemed to be still larger than in those specimens fixed 20 minutes after decapitation.

It is of interest to note that in pieces of tissue fixed within 2 minutes after death, cells with more or less marked post-mortem changes will occasionally be found. Such cells were usually observed singly, but in a few instances several were seen surrounded by or interposed between cells of a normal appearance (Fig. 37, 38). In the latter case, the Golgi apparatus can be observed at the same time in a normal cell and a cell with pronounced post-mortem changes and definite difference in the dimensions of the Golgi vacuoles can be seen. When studying the latter cell at a low magnification, the Golgi vacuoles can only be differentiated with difficulty from the cytoplasm because of cytoplasmic vacuolization.

Discussion.

The experiments carried out here with fixation of tissue taken at varying intervals after death show that changes in the intestinal epithelial cells appear very rapidly. Thus, within 5 minutes after death a partial destruction of both the ground substance of the mitochondria and their inner

membranes is seen, and the mitochondria also show a swelling which manifests itself by a significant increase in width. In the cytoplasm, it is found that some of the normally occurring vacuoles have increased in size. The observed changes are successively accentuated in specimens fixed a longer time after death. In pieces of tissues taken 5 and 10 minutes after decapitation, the Golgi apparatus is found to be of normal appearance when compared with the Golgi apparatus from specimens fixed 2 minutes post mortem, whereas an increase in the size of the vacuoles is observed in specimens fixed 20 and 40 minutes after decapitation. It seems possible that some of the varying descriptions of this cell organelle presented in light microscopic studies may be attributed to this post-mortem increase in the size of the vacuoles. In comparison with the earlier examined proximal tubule cells of the kidney (RHODIN 1954), it is noted that post-mortem changes appear more rapidly in the intestinal epithelial cells. Since the experiments with the kidney cells were carried out at room temperature and those with the intestinal cells at a temperature of $+1^{\circ}$ to $+2^{\circ}\text{C}$, the results are not quite comparable. Yet it seems reasonable to assume that the low temperature would reduce the speed of the post-mortem changes.

Those cells which showed post-mortem changes but had been fixed under optimal conditions might reflect differences in sensitivity to oxygen deficiency, different functional states, age, or various combinations of these factors. These cells may be the same as those described by ZIMMERMANN (1898) as "flaschenförmige, helle Zellen".

This investigation has confirmed earlier observations which have demonstrated that it is essential for the tissues to be immersed in the fixative as soon as possible after the supply of oxygen has been interrupted.

FIXATION AT DIFFERENT OSMOLAR CONCENTRATIONS.

Earlier observations.

A great number of light microscopic studies has been undertaken in order to understand the effect of osmolar concentration in fixation of cells and isolated mitochondria. A survey of these studies has been presented by RHODIN (1954). Most authors seem to consider that an isotonic solution (having an osmotic value identical with that of blood and tissues) is preferable, since they have observed a swelling of both cells and mitochondria in hypotonic solutions and a shrinkage in hypertonic solutions.

PALADE (1952 a, b), SJÖSTRAND (1953 a), RHODIN (1954), and others have studied these problems with the electron microscope. PALADE, who mainly employed a solution of 1 % osmium tetroxide in veronal acetate buffer with a molar concentration of about 0.11, also tried fixation in the same

solution rendered isotonic by the addition of sucrose. He reached the conclusion that cells with a high water content are preserved better in the isotonic solution, although in other cells this condition was not important. A systematic analysis of these conditions was carried out by SJÖSTRAND on the retina of the guinea-pig eye. He found that the ultra-structure was preserved better by fixation in 1 % osmium tetroxide solution buffered with veronal acetate, which had been rendered isotonic with sodium chloride, than by fixation in the same solution without the addition of salt. The isotonic osmium tetroxide solution described by SJÖSTRAND was used by RHODIN in an analysis of the proximal tubule cells of the mouse kidney, in addition to a study of the effect of hypotonic (0.28 M) and hypertonic (0.42 M) fixative solutions. In this analysis, RHODIN noted a tendency towards swelling of mitochondria in hypotonic solution and a corresponding shrinkage in hypertonic solution, although no statistical significance could be demonstrated. As pointed out by RHODIN, the variations in osmolar concentration were fairly small.

J. WEISS (1953) reported a definite swelling of mitochondria after small pieces of mouse pancreas were placed in water for 30 minutes before fixation in an isotonic solution.

Results.

The white mice used in these experiments had been starved for 12 hours and without fluid for 4 hours. In order to check that no post-mortem changes which might appear would be interpreted erroneously as the result of variations in tonicity, samples of tissue from the intestine of the same animal were placed in a hypotonic, a hypertonic and an isotonic buffered (veronal acetate) 1 % osmium tetroxide solution. The difference in time between the immersion of the first sample and the last was about $\frac{1}{2}$ minute. On checking the preparations fixed in the isotonic solution, it was found that in all cases the cells were normal without signs of post-mortem degeneration. The material consisted of three animals from which a total number of 74 cells and about 1,200 mitochondria were studied.

The appearance of the cell structures after fixation in hypotonic (approximately 0.14 M) solution (Fig. 42). In survey pictures it was seen that the opacity of all cells was reduced in comparison with that of cells fixed in the isotonic solution. The decrease in opacity of the specimens varied from animal to animal and was marked in one case (Fig. 42). Specimens from the other two animals presented a less marked but still appreciable decrease in opacity compared to specimens fixed in isotonic solution. This decrease in opacity is probably due to a decrease in the density of the ground sub-

stance and to a typical dispersion of the different cell organelles. An analysis of the structures present in the cells, however, showed that all cell components were structurally normal with the exception of the cytoplasmic vesicles. The mean diameter of these structures is increased from 780 ± 50 Å (SD=170, Tab. 3) in isotonic solution to $1,100 \pm 80$ Å (SD=240, Tab. 3) in this hypotonic solution. The membrane dimensions and width of mitochondria were analyzed and the values obtained agreed well with values obtained from mitochondria fixed in isotonic, buffered osmium solution. The width of the mitochondria was 0.28 ± 0.006 μ (SD=0.034, Tab. 4, 6). The dimensions of the mitochondrial membranes are given in Tab. 5.

In experiments employing different fixative solutions an analysis of mitochondrial width in specimens fixed in a 0.04 M osmium tetroxide solution gave a value of 0.26 ± 0.008 μ (SD=0.026, Tab. 4), as did fixation in 0.56 M solution (Fig. 44).

The appearance of the cell structures after fixation in a hypertonic (approximately 0.56 M) solution (Fig. 43). In comparison with preparations fixed in the isotonic solution, the cells fixed in hypertonic solution showed increased opacity due to an increase in the density of the ground substance and to the tightly packed cell organelles and other cytoplasmic structures. The various cellular structures, however, were found to be unchanged, with the exception of the cytoplasmic vesicles which were barely discernable in sections from specimens fixed in hypertonic solution. Therefore it has not been possible to make any measurements of their diameter. The value obtained for the width of the mitochondria was 0.26 ± 0.006 μ (SD=0.028, Tab. 4, 6). The dimensions of mitochondrial membranes are given in Tab. 5.

Discussion.

This investigation has shown that deviations in the osmotic value of the fixative from that of the blood and tissues will influence the ground substance of the cytoplasm and the cytoplasmic vesicles. Thus, the opacity of the ground substance is reduced in hypotonic and increased in hypertonic solutions, while the vesicles have an increased volume resulting from hypotonic fixation and a decreased volume from hypertonic fixation. The degree of influence varies from animal to animal and may be dependent on cellular salt and fluid balance at the time of fixation. As for the mitochondria, it was found that they were unaffected by hypotonic and hypertonic fixative solutions. Thus, no variations in width of the mitochondria have been observed in these cells. Neither are any changes observed in the mitochondrial ground substance

which has the same opacity in specimens fixed in 0.04 M, 0.14 M or 0.56 M solution. The swelling of the mitochondria in hypotonic solution, which has been reported by many authors, is in all probability due to post-mortem changes. Various investigations carried out on cells from the pancreas, the kidney and the intestine, have shown that post-mortem changes occur very rapidly after the experimental animal has been killed. Whether the reported swelling of the mitochondria in hypotonic solution is due only to post-mortem changes or to the combined effect of such changes and hypotonicity cannot be established at present. However, it seems possible to assume that the osmotic sensitivity which has been demonstrated in light microscopic studies may depend upon a primary post-mortem alteration in the permeability of the outer mitochondrial membrane.

The influence on some of the cell components and their interrelationships which can be observed after fixation in solutions whose tonicity markedly deviates from the osmotic value of blood and which in many cases is marked, necessitates the use of fixative solutions which have the same osmotic value as blood. This has been found to be of importance in studying the intestinal epithelial cells.

Thus, this investigation has confirmed the results obtained by SJÖSTRAND (1953 a) whose analysis of the guinea-pig retinal rods indicated a swelling of the outer segments after fixation in a hypotonic solution as compared with fixation in an isotonic solution.

FIXATION AT DIFFERENT pH.

Earlier observations.

Light microscopic studies concerning the influence of the hydrogen ion concentration of fixatives on the appearance of the cell structures have shown that some structures are preserved better in an acid solution, while others are preserved better after fixation in a neutral medium. For the cell as a whole, a pH between 4 and 5 has been suggested as the most suitable (ZEIGER 1938).

In electron microscopy several different fixative solutions have been tried, and it has been found that acid solutions do not give satisfactory results (PORTER, CLAUDE and FULLAM 1945, DALTON, et al., 1950). Experiments with formalin as a fixative also have shown that better results are obtained in a neutral solution than in an acid one (PORTER 1950, ROZSA and WYCKOFF 1950). PORTER, et al., (1945), in experiments with cells in tissue cultures, found that the best fixations were obtained with the use of osmium tetroxide vapors from a 2 % aqueous solution of this substance. Since fixation of tissue directly in this solution gave poorer results, PALADE (1952 a) tried 1 % aqueous solution of osmium tetroxide to which veronal

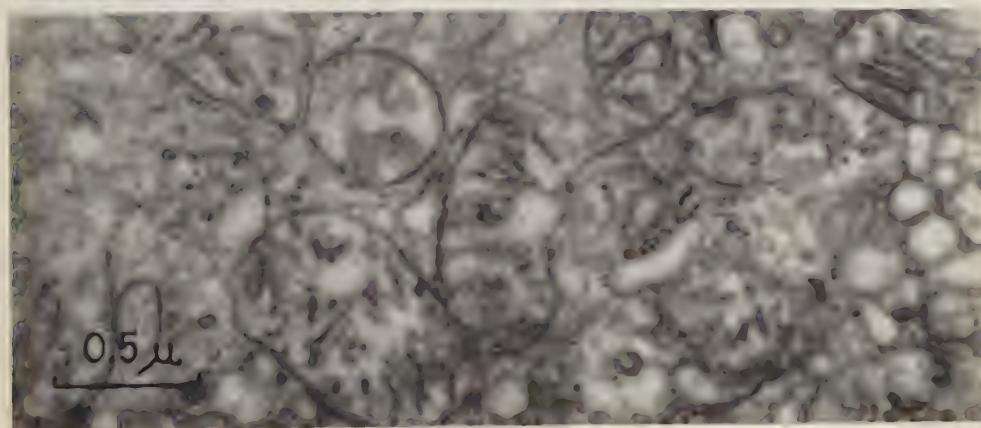
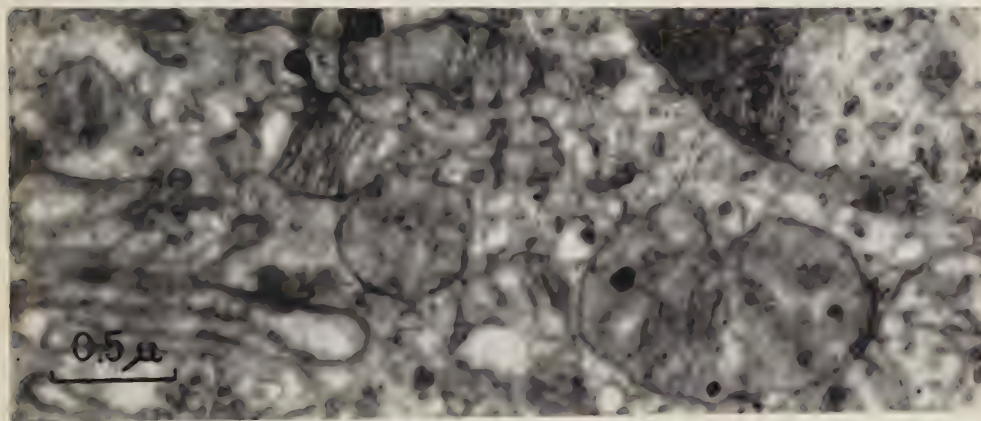
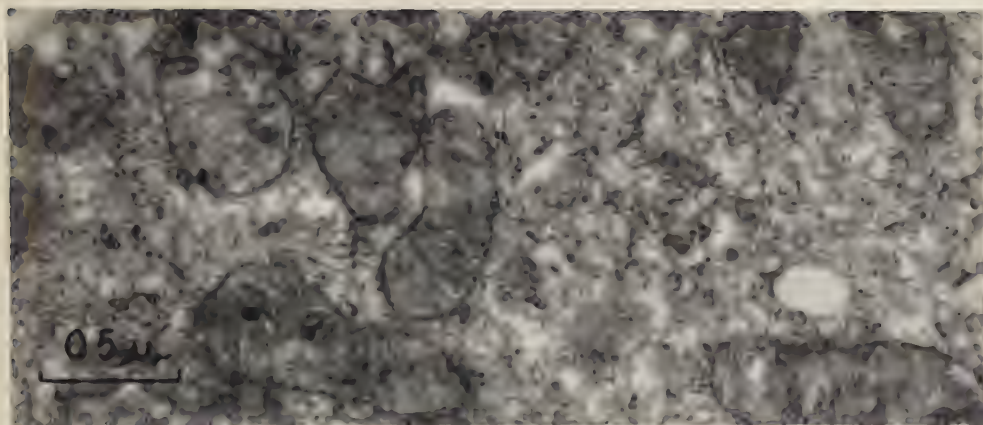


Fig. 34—36. Pictures of post-mortem changes of mitochondria and cytoplasm. Specimens are fixed 5 (top), 10, and 40 (bottom) minutes after decapitation of the mice. The progressive alteration of mitochondria and vacuolization of cytoplasm is observed. Magnification Fig. 34 36,000 $\times$, Fig. 35 32,000 $\times$, Fig. 36 38,000 $\times$.

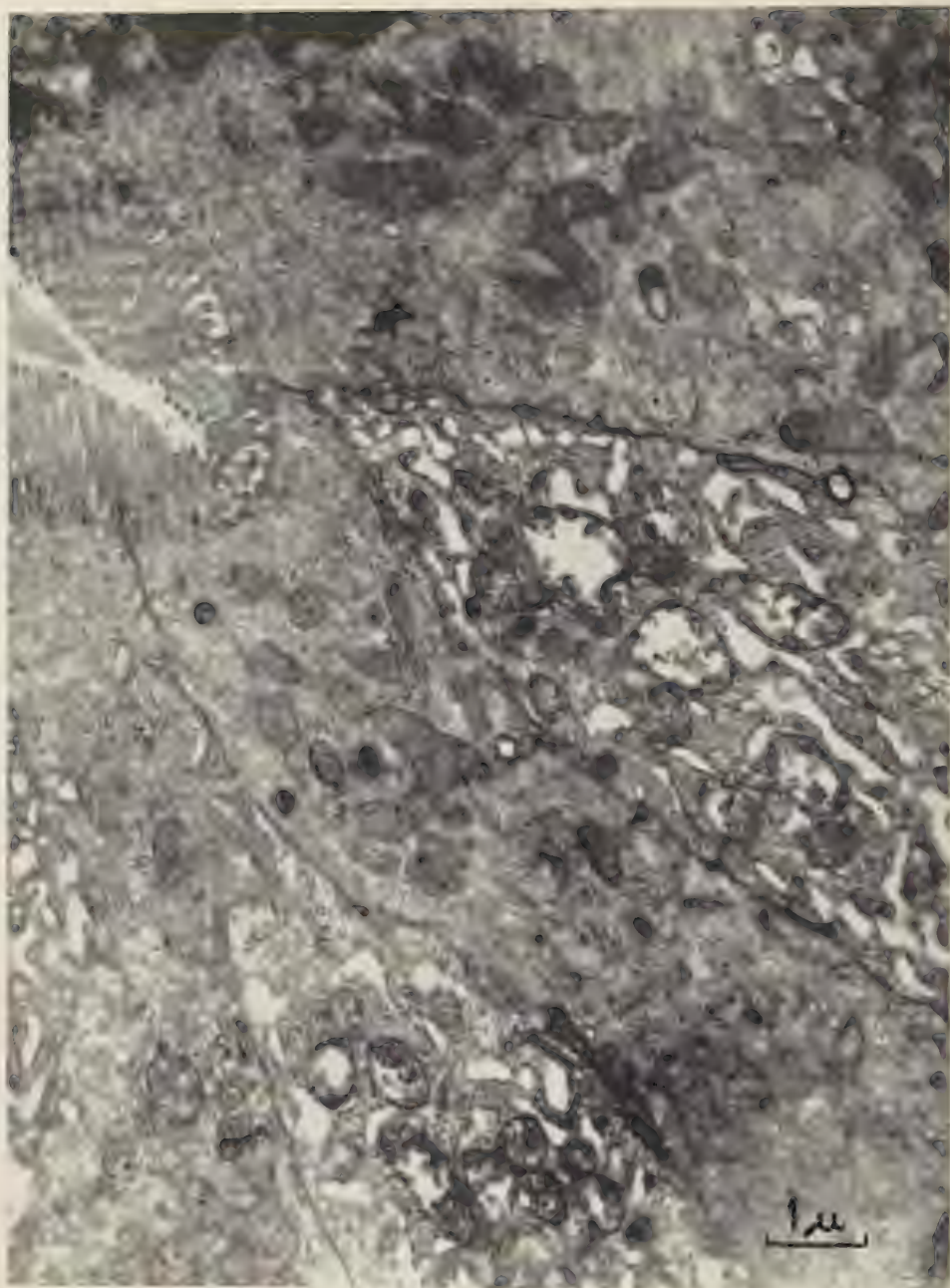


Fig 37. Survey picture of several cells. Every second cell showing changes similar to those described as appearing post mortem with alteration of mitochondria and cytoplasm. Magnification 13,000

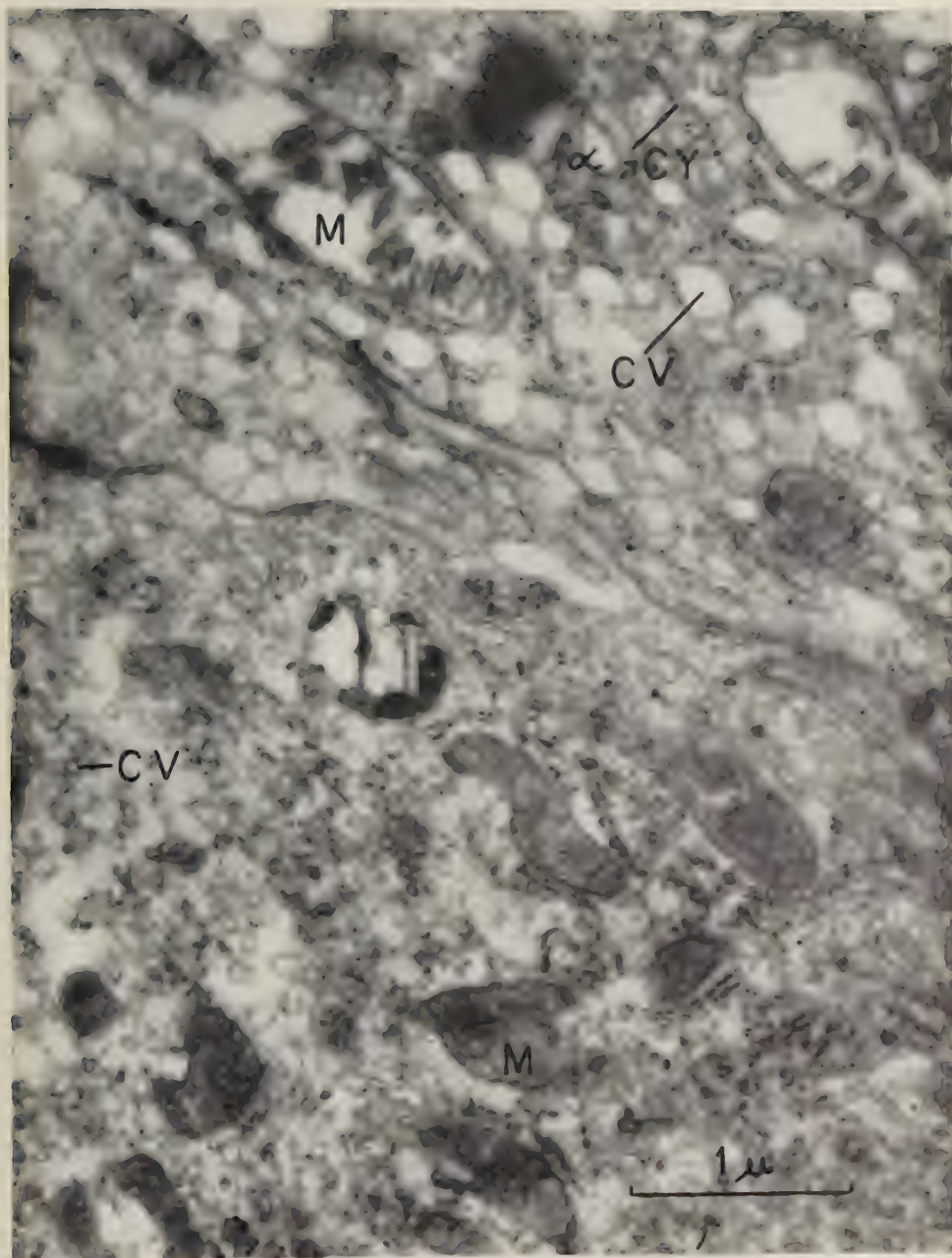


Fig. 38. Section of normal cell (below) and cell with alteration of mitochondria and cytoplasm. In the latter the mitochondria (M) are swollen and show destruction of inner membranes. In the cytoplasm the vesicles (CV) are enlarged and appear empty. The α -cytomembranes (α -cy) appear unchanged. Magnification 30,000 $\times$.

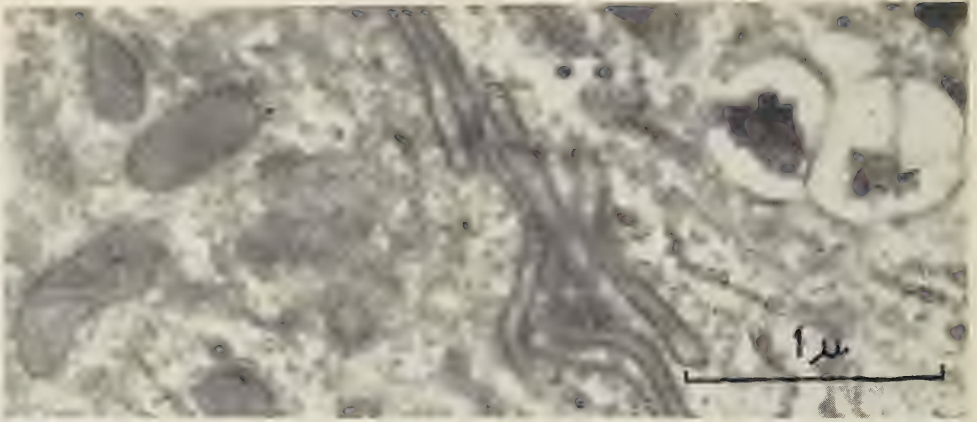


Fig. 39—41. Pictures showing the effect of fixation at different pH. At pH 5.1 (top) the ground substance of the cytoplasm appears vesiculated while the other cell components seem unchanged. At pH 9.5 (bottom) no difference from the neutral pH (middle) is observed.

Magnification Fig. 39 33,000 $\times$, Fig. 40 29,000 $\times$, Fig. 41 32,000 $\times$.

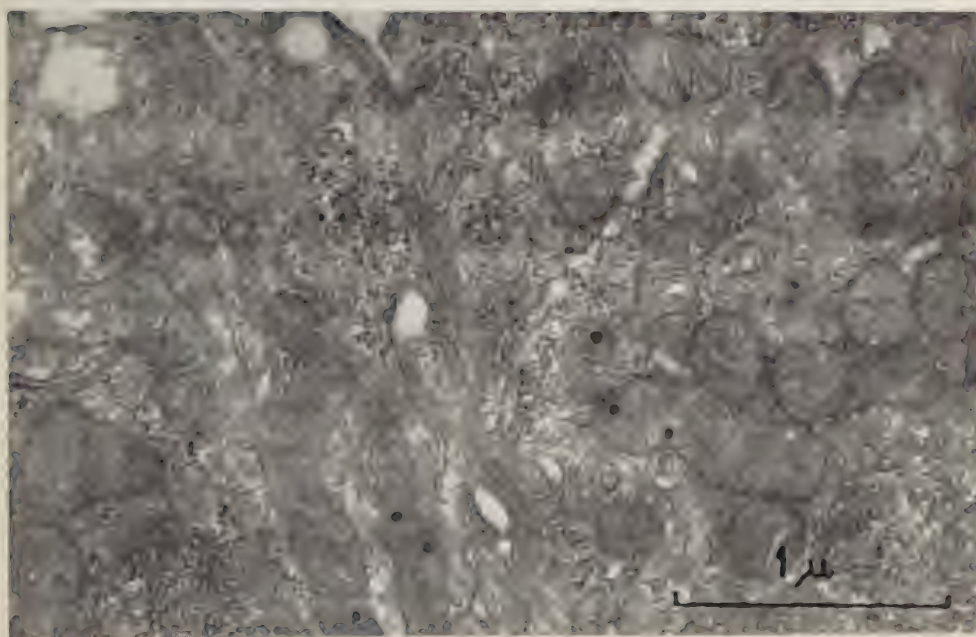
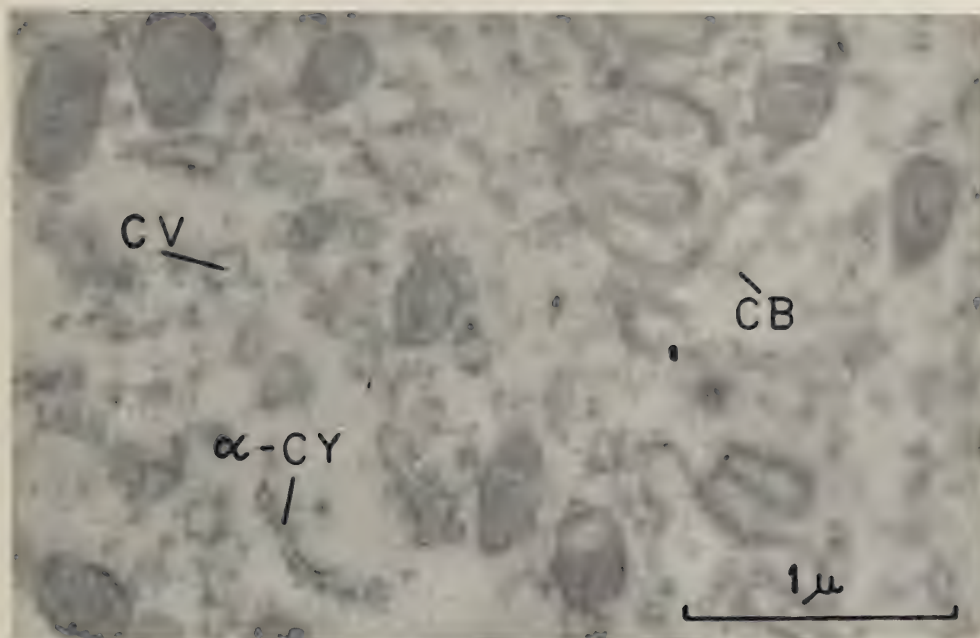


Fig. 42—43. Pictures showing the effect of fixation at different osmolar concentrations. In hypotonic solution (above) the cytoplasm appears empty with long distances between structures and with enlarged cytoplasmic vesicles. In hypertonic solution (below) the different structures are closely packed and no vesicles are observed. In both cases mitochondria appear unchanged.
 Magnification Fig. 42 36,000 $\times$, Fig. 43 37,000 $\times$.

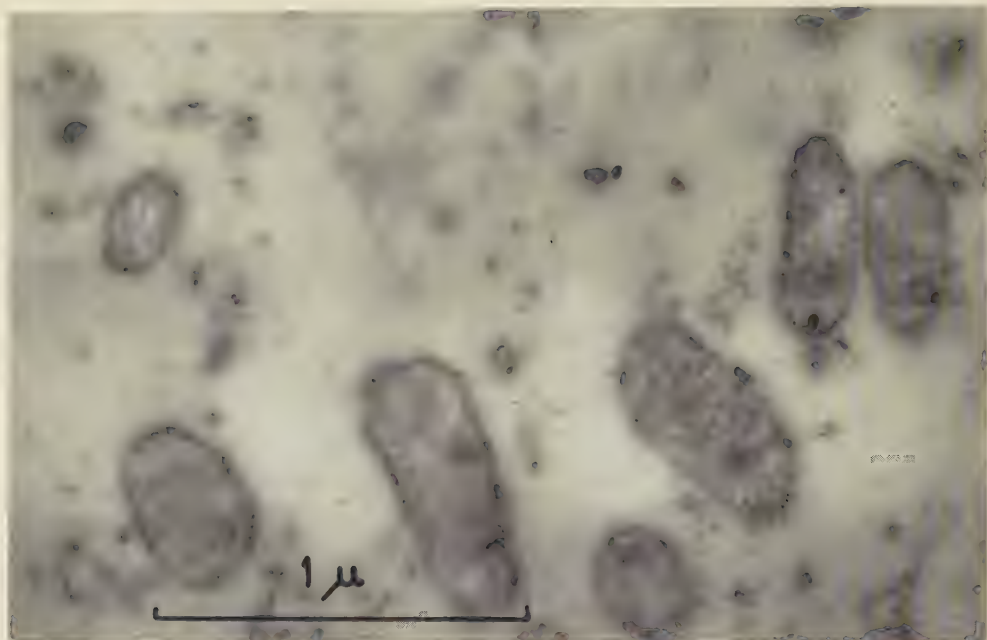


Fig. 44. Section from a cell fixed in a solution of 1 % osmium tetroxide in water with an osmolar concentration of about 0.04 M. In the cytoplasm only few granules and vesicles are observed. The mitochondria appear normal in shape and opacity of the ground substance.
Magnification 49,000 $\times$.

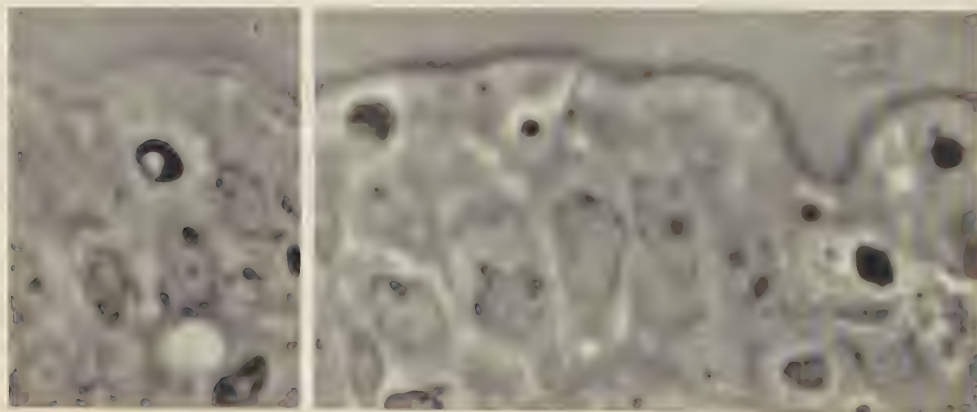


Fig. 45—46. Light micrographs from osmiumfixed and plastic-embedded specimens sectioned at 0.6 μ . Opaque bodies of varying shape and size are seen in the region between brush border and nucleus.
Magnification 1,900 $\times$.

acetate had been added. The pH of this solution was 7.3—7.5. Tissues fixed in this solution were found to be much better preserved. PALADE also carried out studies on tissues fixed at different pH and found that alterations both to the acid and the alkaline side of the above range resulted in poorer fixation with swelling of the mitochondria and vacuolization of the ground substance.

Experiments with fixation at different pH were also carried out by RHODIN (1954). Electron microscopic analysis of kidney tissue after fixation in acid osmium tetroxide solutions (pH 4.5 and 6.2) showed that the tissue was only slightly changed on studying survey pictures with low resolution and magnification. At higher resolution, such changes as vesiculation of the cell cytoplasm and the nucleus, swelling of the mitochondrial membranes, and (at pH 4.5) destruction of the outer mitochondrial membranes were observed. At pH 7.9, on the other hand, the tissue appeared quite normal. Thus, RHODIN's investigation did not confirm PALADE's observations of considerable changes in the cell structures fixed in acid or alkaline solutions. In discussing his observations, RHODIN pointed out that the destruction of the cell structures described by PALADE resembled in several respects alterations in the structure seen in cells in which post-mortem changes had been provoked.

Results.

The animals used in this experiment were white mice. Specimens of the proximal jejunum were taken in the usual way, and different pieces of tissue from the same animal were placed in 1 % veronal acetate buffered, isotonic osmium tetroxide solutions of pH 5.1, 9.5 and 7.2. This order was chosen in order to check that no post-mortem changes which might appear would be interpreted erroneously as the result of the pH of the solution. Examination of the tissue pieces fixed at pH 7.2 showed normal cells in all cases. The experimental series included three animals from which 54 cells and about 850 mitochondria were studied.

The appearance of the cell structures after fixation at pH 5.1 (Fig. 39). Examination of the intestinal epithelial cells showed that changes occurred primarily in the ground substance of the cytoplasm, while the other cellular components were of the same appearance as those fixed at pH 7.2. The degree of influence varied from cell to cell: for instance, the changes were marked in one cell and slight in an adjacent one. In individual cells, the changes seemed to be evenly distributed over the ground substance. In most cases the changes were so marked that they could be distinguished even at low resolution, but occasionally a higher resolution was required

before they could be demonstrated. Analysis of a cell with marked changes showed that the ground substance was largely destroyed by the development of small closely packed vesicle-like structures with low opacity, which were not clearly outlined against one another or against the remaining parts of the ground substance. Between these vesiculated areas were found aggregations of the other cytoplasmic components, which appeared structurally unchanged. They were clearly outlined against the "empty" background with good contrast. The mitochondria were of normal shape, and the value obtained for their width, $0.28 \pm 0.007 \mu$ (SD=0.036, Tab. 4, 7), agreed well with the normal value. The inner and outer membranes appeared predominantly normal but were somewhat vaguely outlined in places. The values obtained for the dimensions of the mitochondrial membranes are given in Tab. 5. The ground substance of the mitochondria showed no changes. The nuclear membrane was found to be unchanged, while the nuclear substance showed a vesiculation comparable to that appearing in the ground substance of the cytoplasm. The brush border with cell membrane and Golgi apparatus were similar in structural detail to those obtained by fixation at pH 7.2.

The appearance of the cell structures after fixation at pH 9.5 (Fig. 41). In analyzing cells fixed in this medium, no definite deviations from the normal structure were demonstrated. The different components in the ground cytoplasm were well preserved, as were the brush border, mitochondria, Golgi apparatus and nucleus. The width of the mitochondria and their membranes was measured. The mitochondrial width was found to be $0.25 \pm 0.007 \mu$ (SD=0.027, Tab. 4, 7). The dimensions of inner and outer membranes are given in Tab. 5.

Discussion.

This investigation has shown that fixation in 1 % isotonic osmium tetroxide solution with pH 5.1 results in poorer preservation when compared with alkaline fixation (pH 9.5) which gives results comparable, on the whole, to those obtained at pH 7.2.

Most of the changes observed in these cells after fixation at pH 5.1 seem to be identical with those described by RHODIN (1954) in the tubule cells of the kidney after fixation at pH 4.5 and 6.2. The destruction of the outer mitochondrial membranes, observed in the cells of the kidney at pH 4.5, on the other hand, are not seen in the preparations from intestinal cells fixed at pH 5.1. However, it seems possible that the vague outlines of the membranes, which appear here and there in these mitochondria, are of the same nature as those observed in the kidney cells. The dif-

ference is probably due to the varying pH employed and to a sensitivity to deviations in the pH of the fixative from neutral values (7.2), which probably varies from cell type to cell type. Regarding the mitochondrial width, this investigation has confirmed RHODIN's observations that the width changes neither in acid nor in alkaline fixatives (Tab. 7). No swelling of the mitochondrial membranes after fixation in acid solution, as reported by RHODIN in his investigation of kidney proximal tubule cells, has been demonstrated in this material.

The conclusion drawn from this investigation is that fixation of intestinal epithelial cells in 1 % veronal acetate buffered, isotonic osmium tetroxide solution at pH 5.1 results in the destruction of the cytoplasmic ground substance, while the same solution at pH 9.5 results in preservation of the tissue comparable to that obtained at pH 7.2.

IV

VITAMIN A DEFICIENCY

Observations made on animals deprived of their supply of vitamin A have led to the conclusion that this vitamin plays a fundamental part in cell metabolism (WALD 1954). Despite extensive research it has not been possible to establish further the function of vitamin A beyond its role in visual processes, even though it has been pointed out that this vitamin is probably important for the maintenance of the mitochondria (WOLBACH 1937).

The development of electron microscopic technique and sectioning methods have made possible cellular analysis on a macromolecular level. Although no changes in the structure of the jejunal epithelium in vitamin A deficiency have been shown by light microscopy these new techniques justify an analysis of this and other epithelial types from animals in which vitamin A deficiency has been induced.

Earlier observations.

Tissue changes as a result of vitamin A deficiency were demonstrated for the first time by MORI (1922) in an investigation of different kinds of epithelium from rats. These animals had been raised on a diet which lacked fat-soluble vitamins. He described the changes as a keratinization and noted the appearance of kerato-hyaline bodies in some tissues. These observations were later confirmed by several workers (WOLBACH and HOWE 1925, GOLDBLATT and BENISCHEK 1927, WOLBACH 1937, 1942). These authors described the changes as metaplasia of the cylindrical or cuboidal or transitional type of epithelium to the squamous keratinizing type. Changes of this type have been shown in various parts of the respiratory and urogenital tract, the cornea, and different glandular epithelium. Regarding the structure of the intestinal epithelium in vitamin A deficiency, CRAMER (1923) described atrophic changes in the lower part of the ileum in rats, while WOLBACH and HOWE (1925), in specimens from man and rats, as well as WOLFE and SALTER (1931), in specimens from mice, did not find any changes in this epithelium.

Material and methods.

A total of 40 white mice weighing between 3 and 10 grams, which were born within one week and taken directly from the mother, were placed in glass jars containing wood-shavings and covered with wire-net. Any

coprophagy that might occur was judged to be unimportant. The diet employed was devised by the Vitamin Department of the National Institute of Public Health (Stockholm, Sweden) and had the following composition:

Wheat starch (heated for an hour in steam at 100°C)	625 gm.
Casein, extracted, vitamin-free (Kabi Company)	180 gm.
Dried brewer's yeast (Kabi Company)	100 gm.
Salts, E. Brunius' mixture No. 5a	40 gm.
Salts, E. Brunius' mixture No. 5b	5 gm.
Peanut oil with vitamin D ₃	50 gm.
	Total 1,000 gm.

Since the peanut oil with vitamin D oil contained 60 I.U. of vitamin D per gram, the vitamin D content of the routine diet amounted to 3 I.U. per gram.

The composition of the salt mixture is shown in Tab. 8 and 9.

Diets and water were provided *ad libitum* in glass containers.

To be sure that any changes which might occur were due only to the lack of vitamin A, supplements of vitamin B₁₂, C, E, K and P were given. The amount of these additional vitamins converted into I.U. per animal and day is shown in Tab. 10. Half the number of animal pairs were controls and were given water-soluble vitamin A* in addition to the vitamins listed above.

Results.

During the course of many weeks, the first generation of experimental animals did not show any symptoms of vitamin A deficiency. They grew as fast as the control animals, and along with these controls gave rise to a second generation with one or two and, in one case, three litters. The first litter of the second generation, in two cases, gave rise to a third generation. The first symptoms of vitamin A deficiency developed between 3 and 4 months, but the last animals in the experimental series survived longer than 8 months.

The symptoms which occurred gradually were the classical retardation of growth in young animals and xerophthalmia with hemorrhagic, incrustated and infected eyes. A considerably high mortality in the experimental animals was interpreted as a sign of increased affinity for infections, which is included among the symptoms. Specimens were taken from the jejunum, cornea, retina and bladder of animals which had developed these symptoms. Light microscopic investigation of the epithelium from the cornea

* Kindly placed at my disposal by AB Astra, Södertälje, Sweden.

and bladder showed conversion into stratified keratinizing epithelium containing so-called kerato-hyaline bodies (SHELDON and ZETTERQVIST 1956 a, b, ZETTERQVIST and SHELDON 1956). Analysis of control animals, which were the same age and killed at the same time, showed normal conditions.

Of the specimens taken from these 16 animals, the jejunal specimens from 8 animals were eliminated, since they showed artefacts typical of poor fixation in the electron microscope. Additional sectioning was done on the specimens from the remaining 8 animals. A tabular description is shown in Tab. 11. The number of cells analyzed exceeded 100.

Table 11. Tabular description of Vitamin A deficient animals.

Animal		Generation and litter	Weight in grams final	General condition	Clinical ophthalmia	Histological keratinization of the cornea	Weight in grams control animals
No.	Sex						
1	♂	F-1, litter 1	15	Fair	Bilateral	+	24
2	♀	F-1, litter 1	16	Fair	Bilateral	+	24
3	♂	F-1, litter 2	11	Good	None	+	23
4	♂	F-1, litter 1	16	Poor	Bilateral	+	24
5	♀	Parent	14	Poor	Bilateral	+	25
6	♂	F-1, litter 2	14	Poor	Bilateral	+	20
7	♂	F-1, litter 1	14	Poor	Bilateral	+	23
8	♂	F-1, litter 1	14	Poor	Bilateral	+	23

By electron microscopical analysis no structural changes in the cells have been shown. The brush border projections seem normal and the cell membrane remains double-contoured. The organization of the mitochondria is similar to that in the normal material, with double-contoured outer membranes and inner membranes transverse to the long axis. The dimensions of the mitochondrial width and membranes are similar to those described for the normal material. Opaque areas can be seen interspersed between the inner membranes, as is also the case in normal material. In no case have mitochondrial changes been seen comparable to those seen in the corneal or bladder epithelium from the same animals (SHELDON and ZETTERQVIST 1956 a, b, ZETTERQVIST and SHELDON 1956). The nucleus and Golgi apparatus seem normal, as is also the case regarding the structures present in the cytoplasm.

Discussion.

The electron microscopical analysis has not been able to show any changes in the ultrastructural organization of the intestinal epithelium from mice showing metaplastic changes in the corneal and bladder epithelium

which are characteristic of vitamin A deficiency. The investigation confirms the light microscopic observations that metaplasia does not occur in this epithelium. Since no changes occur we can only assume with WOLBACH and BESSEY (1942) that "vitamin A is necessary in some chemical processes uniquely related to normal differentiation and life of some cell types".

GENERAL DISCUSSION

The results of these experiments including a description of the various cell structures have been discussed previously. In the present discussion the question of the reliability of osmium tetroxide as a fixing agent from the ultrastructural point of view will be considered, as well as conclusions regarding the absorption pathways in intestinal epithelium to which this investigation has led.

The osmium fixation. Osmium tetroxide, used in light microscopy since the middle of the last century, has been employed as the most suitable fixative for electron microscopy. It combines fixation with electron staining. Light microscopy has demonstrated that this fixative permits a "life-like preservation" (STRANGEWAYS and CANTI 1927) but there has been some doubt regarding its value as a preserving agent for ultrastructural components.

Comparative investigations of osmium-fixed and freeze-dried material are of great interest, since it is improbable that the same types of artefacts should appear in tissues preserved by these two, totally different methods. Thus, in freeze-dried specimens from pancreas cells, SJÖSTRAND (1953 b) has observed the characteristic membrane systems described in osmium-fixed specimens. The same structural organization of mitochondria and other cell components which is obtained after ordinary osmium tetroxide fixation has also been observed in freeze-dried specimens which have been osmium-stained after the freeze-drying.

The opinion that osmium tetroxide preserves for the most part the ultrastructural organization of the cells is given further support by the agreement of polarization optical and electron microscopical observations, which has been obtained for the outer segments of the retinal rods (SCHMIDT 1934—1935), the membranes of the pancreatic cells (SJÖSTRAND 1953 b), and mitochondria (ROLLHÄUSER 1954).

Regarding the artefact problem, the experiments carried out by earlier investigators and also those carried out in this work have shown that the cell structures are relatively stable, and that the changes which usually occur are of a destructive nature. The most important factor in this case seems to be the time which elapses from the interruption of the oxygen supply to the fixation of the tissues.

The measurements made of the different membrane structures have shown only small deviations of means and they also agree well with the values presented by other authors. This agreement in values present

further evidence of the reliability of osmium tetroxide fixation and has been made the basis for interpretation of molecular arrangement. In this regard, SJÖSTRAND (1953 b) has suggested that the structures observed in the mitochondrial membranes correspond to two protein layers separated by a double layer of lipid molecules in agreement with the model of the cell membrane suggested by DANIELLI (1936) by indirect evidence. This interpretation implies that the structures which appear dark are proteins, while the intermediate light layers consists of lipoids. There is no positive proof for this theory, however, since no specific protein stain has been found useful for electron microscopy. It should be mentioned, however, that experiments with phosphotungstic acid in connection with osmium fixation (ANDERSSON 1956) have given a mitochondrial pattern which is identical with that obtained after regular osmium fixation, with the difference that the opaque membranes, which according to the theory should consists of proteins, now seem darker. Thus, they stain identical components in the cells. Osmium tetroxide as well as phosphotungstic acid have been used extensively in studies of collagen and the muscle proteins and in these cases they also stain identical components which obviously are of protein character.

The measurements which have been made on the structures of the plasma membrane of the free cell surface and on the dimensions of the mitochondrial membranes have shown a certain variance when comparing different cells in the same animal. However, the variances are negligible when a comparison is made between series of cells from different animals. These findings indicate a certain individuality in the membrane dimensions of different cells. Whether this represents a chance variability in the osmium staining, or actual variations in membrane dimensions depending upon varying functional states or age of the cells cannot be settled at the present time.

The absorption pathways. The results of this investigation suggest the probable absorption pathways through the jejunal epithelium. In passing from the intestinal lumen into the cells, the substances to be absorbed must penetrate a plasma membrane, which has a total thickness of about 100 Å. This membrane is composed of two components (40 Å wide) which are osmium stained and separated by a structure (25 Å wide) which is not stained by osmium. The substance passing through the membrane must be in molecular form, since the plasma membrane seems continuous without pores or discontinuities. Transport through the upper part of the cell probably proceeds parallel to the long axis depending upon the arrangement of the α -cytomembranes. In the lower part of the cell, the nucleus

causes a considerable reduction in the potential transport area of the cytoplasm. Analyses of fat absorption have also shown that probably only a small part of the fat passes around the nucleus to the basal parts of the cell and from there through the plasma membrane to the submucosa. The greater part of the fat passes instead out into the intercellular space, which is often enlarged to a considerable extent during fat absorption. Those areas of the plasma membrane which face the intercellular spaces, as well as, the basement membrane have a structure similar in appearance to the trilaminar membrane on the free cell surface. Although here, the dimensions of each component of the membrane is only 20—25 Å.

There are two possibilities for the transport of material from the intercellular space to the blood and lymph vessels found in the submucosa. This transport can take place either between the cell membranes of the basal projections of adjacent cells or through these projections, in which latter case the material must pass through the cell membrane twice. In the electron micrographs there are no indications of any free space between the cell membranes of those projections which would be open for a direct communication between the intercellular spaces and the basement membrane.

It also seems possible that part of the absorption from the intestinal lumen can take place intercellularly. Thus, in those areas where two cells lie close together, two opaque layers are observed belonging to the two cells in contact. These layers are separated by a less opaque layer (about 100 Å wide). This layer is not bridged over by any opaque structure in the terminal bar region or at any other place and seems to be in contact with the intestinal lumen as well as the space which often exists basally. Due to the very constant thickness of this intermediate layer it has been interpreted as a component of the cell membrane which is not osmophilic and therefore appears falsely as a free space in the electron micrographs. This interpretation, however, does not exclude the possibility that real spaces separating adjacent cells may appear under certain functional conditions or that certain molecules may diffuse within the non osmophilic layer of the cell membrane. Such assumptions, however, are not yet proven by observations.

Regarding the functional significance of the different cell structures and their importance for absorption, no definite conclusions can be drawn.

SUMMARY

For analysis of the ultrastructural organization of the absorbing cells of the jejunal epithelium from mice, specimens have been fixed at a temperature of $+1^{\circ} - +2^{\circ}\text{C}$ in 1 % veronal acetate buffered, blood isotonic osmium tetroxide solution. After rinsing and dehydration, the specimens were embedded in methachrylate and sectioned with a Sjöstrand microtome employing the sectioning technique developed by SJÖSTRAND. The sections obtained in this way were sufficiently thin to permit routinely a resolution of better than 50 Å. The best resolution achieved is better than 30 Å. The sections were examined in a RCA EMU 2c electron microscope.

Analysis of the brush border has confirmed earlier observations that it is composed of fingerlike projections from the cytoplasm, about $1\ \mu$ long and $0.1\ \mu$ wide. They are closely packed together and number about 600—700 per cell and 50,000,000 per square millimeter of the intestinal surface. In these projections, longitudinal fibrillar structures of very fine dimensions are observed, which extend into the cytoplasm about $\frac{1}{2}\ \mu$ and, in somewhat thicker sections, seem to fuse and in doing so give an impression of a rootlike formation. The brush border projections are covered with a continuous *plasma membrane* composed of two opaque components (about 40 Å in width) bordering a less opaque (about 25 Å thick) layer. In this plasma membrane, no pores or discontinuities are observed. On the surfaces where two cells lie close together, the plasma membranes appear as two opaque lines (about 70 Å wide) separated by a less dark layer about 100 Å in width. Close to the free cell surface the membrane appears to narrow and along its course an area about $1\ \mu$ long can be distinguished from the surrounding cytoplasm as a denser zone representing the terminal bar described in light microscopical studies. Zones of similar construction have also been observed further away from the free cell surface. On the surfaces of the cell adjacent to the space often existing between the bases of the cells and to the basement membrane, the 70 Å wide, opaque component of the plasma membrane is modified to a triple-layered structure, of which two are dense and cover the third which is less dense.

The basement membrane consists of a membrane (about 270 Å wide) of relatively low opacity. This membrane is separated from the cell membrane by a space which, as a rule, is somewhat narrower than the basement membrane.

The rod-shaped mitochondria are found in the supranuclear zones primarily oriented parallel to the long axis of the cell, while those occur-

ing below the nucleus are more irregularly arranged. The mitochondria are delimited by an outer double-contoured membrane and have inner membranes also double-contoured and arranged perpendicularly to the long axis. The contact between the inner and outer membranes seems to be established primarily through the opaque components. Sometimes, however, a direct continuity is observed between the less dense intermediate layers of the inner and outer membranes. There is a statistically significant difference in thickness between the outer and inner membranes, with a mean value of 170 Å for the outer membranes and 210 Å for the inner membranes. Irregularly occurring round, opaque bodies, 200–600 Å in diameter, are seen between the inner membranes. The ground substance of the mitochondria, which appears homogeneous, has a greater density than the surrounding cytoplasm.

The Golgi apparatus, which is found at the distal pole of the nucleus, is composed of groups of membranes, called γ -cytomembranes, and vacuoles embedded in a homogeneous ground substance. These membranes, which have a width of about 60 Å, are arranged in pairs. Approximately 2 to 6 pairs are found, with the ends of each pair fusing together. These membranes referred to as a pair are separated, in places, by vacuoles of varying size containing irregularly formed opaque granules. The distance between adjacent membranes from neighboring pairs is consistently 60 Å. No connections or anastomoses have been observed between the different pairs of membranes. The small vacuoles found in the vicinity of the membranes are often elongated, while those in the remainder of the Golgi zone are more rounded. The diameter of the vacuoles varies between 200 and 400 Å. The membranes delimiting the vacuoles have a thickness of 60 Å. The ground substance, typical for the Golgi zone, seems homogeneous or possibly fine-granulated, with an opacity which is somewhat greater than that of the surrounding cytoplasm.

In the cytoplasm, excluding the mitochondria, Golgi apparatus and structures of similar size, four additional components are observed: a homogeneous ground substance, α -cytomembranes, free granules and vesicles. The ground substance, which appears structureless even at a resolution better than 30 Å, varies in density with the osmotic concentration of the fixative and is vesiculated by acid fixatives. The α -cytomembranes are about 50 Å wide, and occur arranged in pairs delimiting spaces about 300 Å wide. These spaces are arranged primarily in the longitudinal direction of the cell. The distance between the intercommunicating pairs varies up to 3 μ . Irregularly formed granules about 150 Å in diameter are attached to the membranes on the sides not facing the space. Granules of the same

appearance and size as those attached to the α -cytomembranes also occur freely in the cytoplasm and are usually arranged in groups. The fourth component consists of vesicles 300—1,000 Å in diameter, delimited by a membrane about 50 Å wide.

The nucleus, found in the proximal half of the cell is surrounded by two opaque membranes 50 Å wide, which are separated by a less dense layer the width of which varies between 100 and 300 Å. In tangential sections through the membrane, annular-like structures with a diameter of 1,000 Å are observed. These structures probably correspond to the discontinuities observed, on a few occasions, in sections cut perpendicular to the membrane. The outer and the inner membrane seem to fuse on each side of the discontinuities mentioned above. Whether these discontinuities are true holes in the membranes surrounding the nucleus or bridged over by a thin membrane has not been possible to ascertain. A zone (200 Å wide) of increased opacity is observed on both sides of the membrane discontinuities. This zone extends a few hundred Å into the nucleoplasm as well as out into the cytoplasm. The chromatin in the nucleus, which consists of irregularly rounded, opaque granules about 170 Å in diameter, appear fairly evenly distributed throughout the nucleus, with some accumulations towards the periphery. Less dense areas are often observed in these aggregates.

The nucleolus is made up of granules of the same size and appearance as those in other parts of the nucleus, though in the nucleolus the granules are arranged in irregularly intercommunicating rows, with a width of 0.1—0.2 μ .

A structure, not previously described in electron microscopical investigations, is found in the area between the brush border and the nucleus. The size of this structure varies between 0.25 and 4 μ . It is surrounded by a double-contoured membrane and has a ground substance of the same appearance as that of the mitochondria. Opaque granules, with a diameter of 60 Å are seen in this structure. These granules appear irregularly distributed or arranged in groups or rows. Furthermore, membrane structures are seen, which are sometimes double-contoured and have the same dimensions as mitochondrial membranes. Connections have been observed between the granular rows and the membrane structures. Irregularly shaped "empty" areas of varying size have also been observed in this cell component. These structures have also been seen in light microscopic studies of osmium fixed specimens. A comparison has been made between this structure and the cell center seen in material stained with iron-hematoxylin by light microscopy. It seems probable that these two structures are identical. In this regard, the accumulation of small granules arranged

in rows or groups would correspond to one diplosome component, while the rest of the structure would correspond to the light zone seen in light microscope studies.

Another cell component which was observed is called *vacuole-containing body*. It is characterized by relatively close arrangement of small vacuoles (400 Å in diameter) surrounded by a membrane 50 Å wide. This structure appears to be identical with a structure which has shown a positive reaction for acid phosphatase in a combined histochemical and electron microscopical analysis.

Post-mortem changes. Specimens have been taken 5, 10, 20 and 40 minutes after decapitation. In 5 minutes, post-mortem changes in the mitochondria can be observed in the form of increasing width and alterations of the inner membrane system. Furthermore, single enlarged cytoplasmic vesicles can be seen which have diameters up to 0.2 μ . These changes are accentuated successively in specimens fixed a longer time after death. Thus, after 40 minutes, the inner membranes of the mitochondria are largely destroyed and considerable vacuolization of the cytoplasm can be seen.

Tonicity variations. As a result of fixation in hypotonic solution (estimated molar concentration 0.14 M), the opacity of the cell appears lower than that resulting from fixation in isotonic solution. A hypertonic solution (0.56 M), on the other hand, results in an increased density. These changes are probably conditioned by changes in the water content of the cell. Regarding the different cell structures it is observed that the cytoplasmic vesicles swell in hypotonic and shrink in hypertonic solution, while the other cell components appear unaffected. The mitochondria have been analyzed regarding their width and membrane dimensions and the values obtained agree well with each other and with the values from the cells fixed in isotonic solution.

pH-variations. This investigation shows that fixation in acid solutions affects the ground substance of the cytoplasm, where small vesicle-like structures with low density are observed and between which the other cell components are aggregated. The cell components seem otherwise unaffected. Fixation at pH 9.5 produces results which are comparable to those obtained at pH 7.2.

Vitamin A deficiency. A completely normal ultrastructure was found in a study of intestinal epithelial cells from mice in which changes typical for vitamin A deficiency had been observed in the corneal and bladder epithelium.

TABLES

Table 1. The result of analysis of variance performed on measurements of the plasma membrane covering the brush border projections.

Standard deviation between	Total thickness	The distance between the centers of the two opaque layers
Animals	0	1.8
Cells	12.1	5.1
Projections within the same cell	5.8	2.9

Table 2. The results of analysis of variance performed on measurements of the mitochondrial outer and inner membranes.

Standard deviation between	Outer membranes		Inner membranes	
	Total thickness	The distance between the centers of the two opaque layers	Total thickness	The distance between the centers of the two opaque layers
Animals	0	0	0	1.9
Cells	17.9	13.1	181.4	13.7
Mitochondria within the same cell	5.0	5.2	3.1	2.0

Table 3. The number of measurements performed on different cell structures.

Structures	Number of performed measurements	Number of investigated	
		Cells	Animals
<i>Brush border extensions</i>			
Length	150	15	11
Width	750	15	11
Number per μ^2	—	13	10
<i>Plasma membrane on the free cell surface</i>			
Total thickness	361	7	5
Distance between the centers of the two opaque layers	361	7	5
<i>Plasma membrane between cells lying close together</i>			
Total thickness	130	13	10
Distance between the centers of the two opaque layers	130	13	10
<i>Basement membrane</i>			
Width	110	11	6
<i>Golgi membranes</i>			
Total width of a false pair	122	10	8
Distance between the centers of the two opaque layers	122	10	8
<i>Cytoplasm</i>			
<i>α-cytomembranes</i>			
Thickness	200	20	15
Distance between the components of a pair	400	20	11
Diameter of attached granules	200	20	11
<i>Free granules</i>			
Diameter	360	18	10
<i>Vesicles</i>			
Diameter	100	10	6
<i>Nucleus</i>			
Outer membrane	80	8	7
Inner membrane	80	8	7
Granules	100	10	8
<i>Cell component I</i>			
Small granules	160	8	8
<i>Vacuole-containing body</i>			
Thickness of outer membrane	110	11	8
Diameter of small vacuoles	110	11	8
<i>Cytoplasmic vesicles</i>			
Diameter after fixation in 0.14 M fixative solution	100	10	6

Table 4. The width of mitochondria in cells fixed under different experimental conditions.

Experimental conditions	Width in μ		Number of mitochondria	Number of cells	Number of animals
	Mean	SD			
Fixation within 2 min. after decapitation. 0.34 M, pH 7.2	0.28 ± 0.006	0.029	1,267	72	18
Fixation 5 min. after decapitation. 0.34 M, pH 7.2	0.36 ± 0.006	0.031	429	29	4
Fixation 10 min. after decapitation. 0.34 M, pH 7.2	0.37 ± 0.009	0.038	291	18	2
Fixation 20 min. after decapitation. 0.34 M, pH 7.2	0.39 ± 0.019	0.060	155	10	2
Fixation 40 min. after decapitation. 0.34 M, pH 7.2	0.47 ± 0.016	0.059	116	10	2
Fixation within 2 min. after decapitation. 0.04 M, pH 7.2	0.26 ± 0.009	0.026	108	9	2
Fixation within 2 min. after decapitation. 0.14 M, pH 7.2	0.28 ± 0.006	0.034	478	31	3
Fixation within 2 min. after decapitation. 0.56 M, pH 7.2	0.26 ± 0.006	0.028	302	20	3
Fixation within 2 min. after decapitation. 0.34 M, pH 5.1	0.28 ± 0.007	0.036	354	25	3
Fixation within 2 min. after decapitation. 0.34 M, pH 9.5	0.25 ± 0.007	0.027	226	13	3

Table 5. Dimensions of the mitochondrial membranes.

Experimental conditions	The total thickness in Å	The distance between the centers of the two opaque layers in Å	Calculated thickness of the single opaque layer in Å	Calculated width of the space between the two opaque layers in Å	Number of per-formed measurements	Number of investigated		
						Mito-chond-ria	Cells	Animals
Fixation within 2 min. after decapitation. 0.34 M, pH 7.2	210	140	65	75	519	38	22	14
	170	110	60	55	165	28	16	13
Fixation 5 min. after decapitation 0.34 M, pH 7.2	210	140	70	70	90	9	9	4
	170	110	55	50	90	9	9	4
Fixation 40 min. after decapitation 0.34 M, pH 7.2	190	130	60	70	70	7	7	2
	160	100	60	45	70	7	7	2
Fixation within 2 min. after decapitation. 0.14 M, pH 7.2	210	140	65	75	110	11	11	3
	160	100	55	50	110	11	11	3
Fixation within 2 min. after decapitation. 0.56 M, pH 7.2	200	140	65	70	110	11	11	3
	160	110	55	50	110	11	11	3
Fixation within 2 min. after decapitation. 0.34 M, pH 5.1	190	130	60	75	100	10	10	3
	160	110	55	50	100	10	10	3
Fixation within 2 min. after decapitation. 0.34 M, pH 9.5	190	130	60	65	70	7	7	3
	160	110	50	55	70	7	7	3

Table 6. The width of mitochondria in cells fixed in solutions of different osmolar concentrations.

Animal number	0.14 M					0.34 M					0.56 M				
	Width in μ			Number of		Width in μ			Number of		Width in μ			Number of	
	Mean	SD		Cells	Mito-chondria	Mean	SD		Cells	Mito-chondria	Mean	SD		Cells	Mito-chondria
1	0.25 ± 0.007	0.024		13	208	0.29 ± 0.008	0.019		6	117	0.26 ± 0.003	0.009		7	115
2	0.29 ± 0.008	0.023		8	117	0.31 ± 0.004	0.013		9	136	0.25 ± 0.004	0.009		6	79
3	0.31 ± 0.007	0.021		10	153	0.24 ± 0.010	0.015		2	25	0.26 ± 0.003	0.007		7	108

Table 7. The width of mitochondria in cells fixed in solutions of different pH.

Animal number	pH 5.1					pH 7.2					pH 9.5				
	Width in μ			Number of		Width in μ			Number of		Width in μ			Number of	
	Mean	SD		Cells	Mito-chondria	Mean	SD		Cells	Mito-chondria	Mean	SD		Cells	Mito-chondria
1	0.31 ± 0.008	0.025		11	141	0.27 ± 0.015	0.037		6	88	0.25 ± 0.008	0.016		4	73
2	0.27 ± 0.005	0.016		11	162	0.27 ± 0.007	0.017		6	101	0.24 ± 0.005	0.012		6	114
3	0.23 ± 0.005	0.010		3	51	0.28 ± 0.012	0.024		4	82	0.26 ± 0.033	0.057		3	39

Table 8. Mixture of Salts No. 5 a.

KH_2PO_4	384.00000 gm.
CaCO_3	405.80000 gm.
KCl	28.04000 gm.
NaCl	84.05000 gm.
$\text{MgSO}_4 + \text{H}_2\text{O}$	56.91000 gm.
$\text{FeSO}_4 + 7 \text{H}_2\text{O}$	31.12000 gm.
$\text{MnSO}_4 + 4 \text{H}_2\text{O}$	8.12000 gm.
$\text{CuSO}_4 + 5 \text{H}_2\text{O}$	0.98200 gm.
$\text{ZnSO}_4 + 7 \text{H}_2\text{O}$	0.54980 gm.
$\text{CoCl}_2 + 6 \text{H}_2\text{O}$	0.02019 gm.
$\text{KAl}(\text{SO}_4)_2 + 12 \text{H}_2\text{O}$	0.08795 gm.
NaF	0.27630 gm.
KBr	0.01861 gm.
Na_2HAsO_3	0.01134 gm.
$\text{Na}_2\text{B}_4\text{O}_7 + 10 \text{H}_2\text{O}$	0.02203 gm.

Table 9. Mixture of Salts No. 5 b.

NaCl	124.18000 gm.
KI	0.81800 gm.

Table 10. Vitamin supplement.

Vitamin B ₁₂ —	0.3 γ /animal/day	(cyanocobalamine, Behepan*, Kabi)
Vitamin C —	3.5 mg./animal/day	(ascorbic acid)
Vitamin E —	0.3 mg./animal/day	(α -tocopherol, E-vimin*, Astra)
Vitamin K —	0.15 mg./animal/day	(menadiol tetrasodium phosphate, K-vimin*, Astra)
Vitamin P —	0.7 mg./animal/day	(rutin, Ruvitil*, Leo)
Vitamin A** —	5.0 I.U./animal/day	(water soluble, A-vimin*, Astra)

* Proprietary name.

** Control animals only.

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